PB-217 419

TRANSPORT AND RETENTION OF VIRUS-SIZED PARTICLES IN POROUS MEDIA

R.W. Filmer, et al

Colorado State University
Fort Collins, Colorado

December 1965

DISTRIBUTED BY:

NTIS
National Technical Information Service
U. S. DEPARTMENT OF COMMERCE
5285 Port Royal Road, Springfield Va. 22151

WP00349-01

PB 217 419

TRANSPORT AND RETENTION OF VIRUS-SIZED PARTICLES IN POROUS MEDIA

by
R. W. Filmer
and
A. T. Corey

Prepared for
U. S. Department of Health, Education and Welfare
Public Health Service
Grant WP-00349-01

Reproduced by
NATIONAL TECHNICAL
INFORMATION SERVICE
U S Department of Commerce
Springfield VA 22151

Agricultural Engineering Department
Colorado State University
Fort Collins, Colorado
December 1965

130

TRANSPORT AND RETENTION OF VIRUS-SIZED PARTICLES IN POROUS MEDIA

by
R. W. Filmer
and
A. T. Corey

Prepared for
U. S. Department of Health, Education and Welfare
Public Health Service
Grant WP-00349-01

Agricultural Engineering Department
Colorado State University
Fort Collins, Colorado
December 1965

ACKNOWLEDGEMENTS

The authors express their appreciation to the Public Health Service for the grant which made this study possible.

Thanks are due John Borrelli for assistance in the laboratory and also W. A. Norvell and C. A. Bower of the U. S. Salinity Laboratory for specific surface measurements. Thanks are also due Jerrell Fair for preparing and analyzing bacteriophage cultures and L. G. King for furnishing sketches of permeameter components.

Gratitude is extended to Hanae Akari and Theodore Conti for drafting work, to Shih-tun Su for assistance in preparing the figures, to Duayne Barnhart for technical photography, to Roberta Campen and Lilah Roberts for reproduction of this manuscript and to Preston Cobb, Ralph Asmus and other members of the Colorado State University hydraulic laboratory machine shop for cooperation in the design and fabrication of special equipment.

Sincere appreciation is given by the authors to Mrs. Linda Counter for typing the final manuscript in addition to numerous rough drafts.

TABLE OF CONTENTS

Chapter Page

I. INTRODUCTION 1

II. REVIEW OF LITERATURE 4

A. Properties of Protein Particles 4

1. Physical 4
2. Chemical 5
3. Electrokinetic and surface 6

B. Flow in Porous Media 6

C. Retention of Small Particles in Porous Media 9

1. Retention of inorganic particles . . 9
2. Retention of microorganisms (bacteria-sized) 11
3. Retention of virus-sized particles 14

D. Mathematical Analysis of Filtration . . 16

III. THEORY 19

A. Analysis of Removal 20

1. Removal by sieving 22
2. Removal by adsorption 30

IV. PROCEDURE AND EXPERIMENTAL EQUIPMENT 36

A. Hydraulic Equipment 37

1. Permeameter sections 37
2. Assembling the permeameters . . . 41
3. Packing 41
4. Saturation of the soil 44
5. Setting up for the run 44
6. Establishing hydraulic equilibrium. 45

Table of Contents - cont.

Chapter Page

B. Materials Used in Transport Studies . 45

1. Media 45
2. Selection of a virus-sized particle 47
3. Description of protein materials. . 51

C. Procedure for Studying Protein Infiltration 52

1. Introduction of the particles 52
2. Tracing the particles 53

D. Procedural Modifications 54

1. Change of soil structure 54
2. Pre-treatment of soil surface . . . 56
3. "Exchange phenomena" 56

E. Supplementary Experiments 57

1. Correlation of retentive capacity with specific surface area 57
2. Hydraulic properties of materials. 58

F. Precautions 58

V. RESULTS AND CONCLUSIONS 60

A. Discussion of Experimental Results . . 60

1. Evidence contradicting the sieving theory 61
2. Evidence supporting the adsorption theory 65

B. Conclusions 88

C. Recommendations 89

BIBLIOGRAPHY 91

Table of Contents - cont.

Chapter Page

APPENDICES

A. Bacteriophage Labeling with Radioactive Iodine 96

B. Relative Permeability-Capillary Pressure Data 97

C. Capillary Pressure-Saturation Data . . 98

D. Calculation of the Albumin Retained in the Permeameter 101

E. Data for Distribution of Retained Albumin in Soil Columns 102

LIST OF FIGURES

Figure No.	Title	Page
1	Comparison of particle sizes	2a
2	Acrylic components of permeameters	38
3	Tensiometers used for measuring capillary pressure	39
4	Assembled permeameter	42
5	Inflow barrier assembly	43
6	Apparatus for studying the transport of radioactive protein particles	46
7	Sieve Analyses for Greeley sand and White sand	48
8	Comparison of data for the distribution of albumin obtained by scanning with that obtained by sectioning	62
9	Distribution of albumin retained in a soil column	64
10	Distribution of albumin retained in a soil column	68
11	Distribution of albumin retained in a soil column	69
12	Distribution of albumin retained in a soil column	70
13	Distribution of albumin retained in a soil column	71
14	Distribution of albumin retained in a soil column	72
15	Distribution of albumin retained in a soil column	73
16	Distribution of albumin retained in a soil column	76

List of Figures - cont.

Figure No.	Title	Page
17	Distribution of albumin retained in a soil column	77
18	Distribution of albumin retained in a soil column	78
19	Distribution of albumin retained in a soil column	79
20	Distribution of albumin retained in a soil column	80
21	Distribution of albumin retained in a soil column	81
22	Distribution of albumin retained in a soil column	86
23	Relative permeability as a function of capillary pressure	97
24	Saturation as a function of capillary pressure	99
25	Apparatus for measuring saturation as a function of capillary pressure	100

LIST OF TABLES

Table No.	Title	Page
1	Proteinaceous Particles - Size and Shape	4a
2	Distribution of retained material subsequent to separation of sand grains and repeated washing	66
3	Effect of Pre-Treatment on Retention of Labeled Protein.	75
4	Retentive Capacity and Specific Surface .	83
5	Effect of Soil Type on Total Retention $S_e = 1.0$	84
6	Effect of Degree of Saturation on Total Retention	87

LIST OF SYMBOLS

Symbol	Meaning	Dimensions
a	empirical constant	none
A	cross-sectional area of permeameter	L^2
b	constant related to the heat of adsorption	none
B	constant of integration	none
C	concentration of protein in solution	none
C_o	concentration at $y = 0$	none
d_p	effective diameter of protein particles	L
D	empirical constant -	none
e	subscript meaning effective	none
g	acceleration due to gravity	LT^{-2}
G	constant of integration	none
k	proportionality constant	none
k	shape factor	none
K_w	intrinsic permeability, maximum liquid permeability when the soil is fully saturated	L^2
K_{ew}	effective permeability, permeability to water when both water and air are present in the soil in two distinct phases	L^2
K'_{ew}	effective permeability for pore spaces smaller than the protein particles	L^2
K_{rw}	$\frac{K_{ew}}{K_w}$	none
K'_{rw}	$\frac{K'_{ew}}{K_w}$	none
m	$\frac{\rho_b}{w\phi S\rho}$	L^{-1}

List of Symbols - cont.

Symbol	Meaning	Dimensions
n	empirical constant	none
P^*	piezometric pressure; the quantity $P + \gamma y$	$ML^{-1}T^{-2}$
P_b	bubbling pressure, approximately the minimum capillary pressure on the drainage cycle, at which a continuous air-phase exists in a porous medium	$ML^{-1}T^{-2}$
P_c	capillary pressure, the pressure difference between the air and the water in the pore spaces $P_{air} - P_{water}$	$ML^{-1}T^{-2}$
P_c'	capillary pressure during the drainage cycle corresponding to a degree of saturation at which only those pores smaller than the protein particles are filled with water	$ML^{-1}T^{-2}$
q	volume flux Q/A	LT^{-1}
q'	volume flux through pores smaller than the protein particles	LT^{-1}
Q	steady volumetric flow rate of water or protein suspension	L^3T^{-1}
Q'	volumetric flow rate through pores smaller than the protein particles	L^3T^{-1}
r	a radial distance	L
r_e	effective radius of pores smaller than the protein particles	L
$\overline{R^2_w}$	the mean value of the square of the hydraulic radius for channels in a porous medium	L^2
S	degree of saturation, the ratio of the volume of water to the volume of interconnected pore-space in a bulk element of a porous medium	none

List of Symbols - cont.

Symbol	Meaning	Dimensions
S_r	the residual saturation, a saturation at which K_{ew} is assumed to be zero	none
S_e	effective saturation given by $\frac{S - S_r}{1 - S_r}$	none
t	time	T
T	tortuosity, squared ratio of the distance traveled by fluid elements in going from one point to another, to the actual distance between the two points	none
$\overline{u}$	mean velocity of fluid elements	LT^{-1}
v_y	velocity of the protein slug or wafer	LT^{-1}
w	thickness of the protein wafer	L
w	subscript meaning water or wetting-phase	none
y	depth from the soil surface	L
α	$\frac{1}{n - 1}$	none
β	$\left[\frac{1 - n}{n} a^n m\right]^{\frac{1}{n-1}}$	none
γ	specific weight of water equal to ρg	$ML^{-2}T^{-2}$
η	$2 + 3\lambda$, also the exponent in the equation $K_{rw} = \left(\frac{P_b}{P_c}\right)^{\eta}$	none
θ	concentration of protein retained by the soil in gms. of protein per gm. of soil	none
θ_o	concentration of retained protein at the soil surface, i.e., at $y = 0$	none
$\theta_{max.}$	maximum value of the concentration of protein retained by the soil	none

List of Symbols - cont.

Symbol	Meaning	Dimensions
λ	exponent in the equation $S_e = \left(\frac{P_b}{P_c}\right)^{\lambda}$, called the pore-size distribution index	none
μ	dynamic viscosity of water	$ML^{-1}T^{-1}$
ζ	contact angle of water at a solid interface	none
ρ	density of water	ML^{-3}
ρ_b	bulk density of the soil	ML^{-3}
σ	interfacial tension	MT^{-2}
ϕ	porosity, volume of pore space expressed as a fraction of the bulk volume of a porous medium	none
ψ	ratio of the velocity of the protein wafer to the mean velocity of the fluid elements	none
I. H. S. A.	Radioiodinated Human Serum Albumin	

CHAPTER 1

INTRODUCTION

Water, essential to life, also plays a leading role in the transmission of human diseases. As population increases, the danger from water-borne diseases is intensified.

Pathogenic organisms (bacteria and viruses) are often transported by water in open channels and through the ground. Viruses being much smaller than bacteria, are much more difficult to remove from water by filtration and the danger of contamination of ground water by viruses is, therefore, greater than the danger of contamination by bacteria.

Viruses are more difficult to trace than bacteria and consequently, fewer studies have been conducted on the transport of virus particles through soil. In recent years, however, outbreaks of infectious hepatitis have been strongly correlated with ground water contamination (Mosley, 1959, and McLean, 1964). Infectious hepatitis is caused by a virus, and its prevalence is increasing everywhere. It seems appropriate, therefore, that the study of transport of virus particles through soil should be intensified.

A substantial number of studies of the retention of viruses in sand and other types of filters has been conducted. Little is known about virus movement and retention in soil materials under natural conditions, the hydraulic conditions being very different from those encountered in filter beds.

One significant difference in hydraulic conditions encountered in filter beds from those encountered in the vicinity of a leaching pit, for example, is that the hydraulic gradient in the latter case is much less. Furthermore, flow in the vicinity of a leaching pit may occur through partially saturated soil.

It has been only in recent years that knowledge of flow through partially saturated soil has developed sufficiently to permit the formulation of more than qualitative ideas about this type of flow. Now that such knowledge has been developed, it should be possible to develop an understanding of the transport of virus-sized particles through soil under a wider variety of hydraulic conditions.

Although virus particles are very small compared to most bacteria they exist in a wide range of sizes as Figure 1 shows. They vary also with respect to other physical and chemical properties. It is probable, therefore, that the mechanism of retention of viruses in soil materials and the characteristics of their transport through soils vary greatly for different types of viruses.

It is not likely, therefore, that one could extrapolate knowledge of transport phenomena associated with one type of virus to apply directly to the transport of another type of virus. Furthermore, most types of viruses are difficult to obtain (in their natural condition) in sufficient concentrations for use in transport studies.

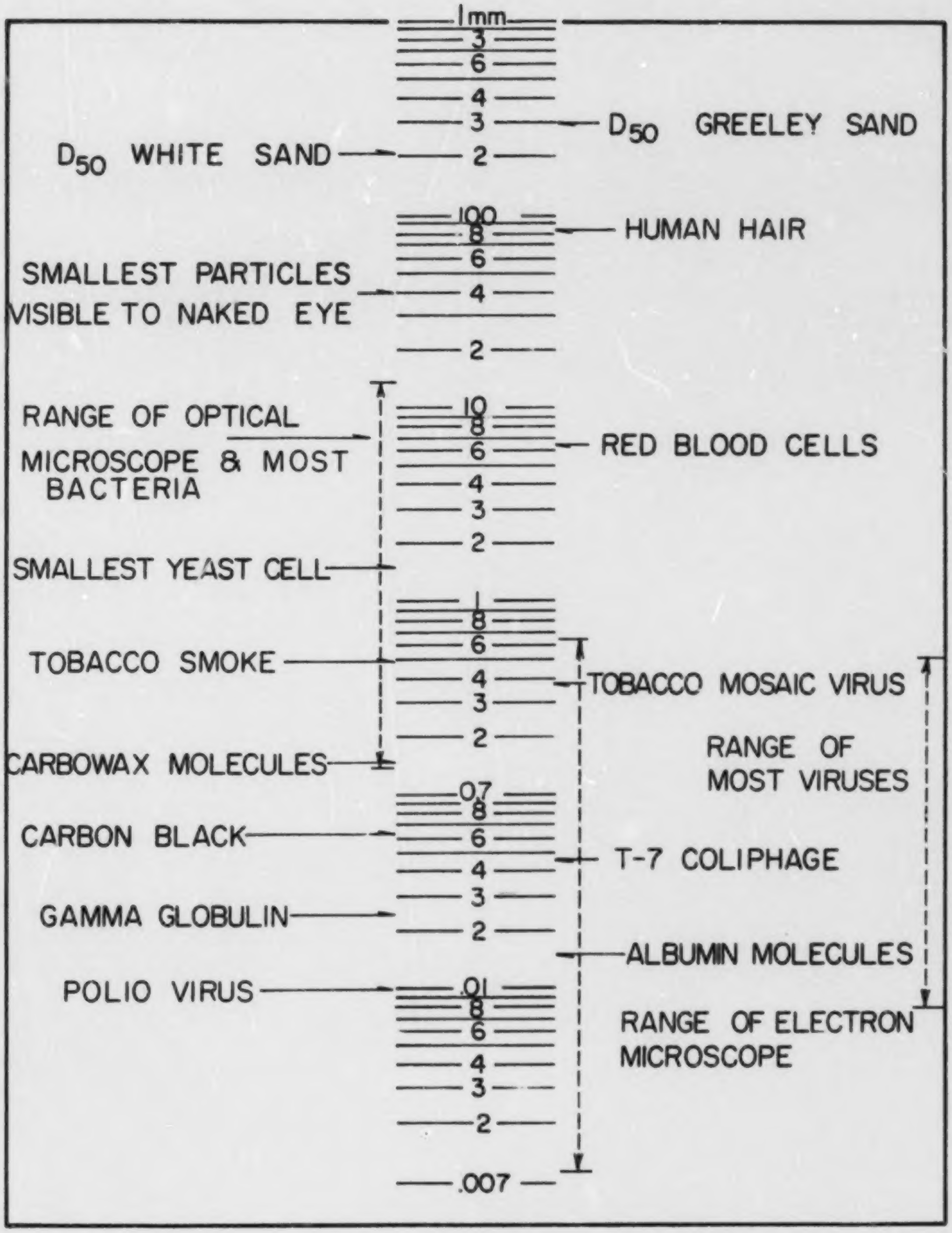

1mm
3
6
4
3
2
100
8
6
4
2
10
8
6
4
3
2
1
8
6
4
3
2
.07
8
6
4
3
2
.01
8
6
4
3
2
.007
D50 WHITE SAND
D50 GREELEY SAND
HUMAN HAIR
SMALLEST PARTICLES VISIBLE TO NAKED EYE
RANGE OF OPTICAL MICROSCOPE & MOST BACTERIA
RED BLOOD CELLS
SMALLEST YEAST CELL
TOBACCO SMOKE
TOBACCO MOSAIC VIRUS
RANGE OF MOST VIRUSES
CARBOWAX MOLECULES
CARBON BLACK
T-7 COLIPHAGE
GAMMA GLOBULIN
ALBUMIN MOLECULES
POLIO VIRUS
RANGE OF ELECTRON MICROSCOPE

Figure 1. Comparison of particle sizes

In this investigation, most of the studies were conducted using albumin particles. Albumin, like viruses, are proteinaceous particles. The size of albumin particles is within the range of the smaller viruses, and it is expected that their physical and chemical properties may be as representative of this class of particles as any particular type of virus. In any case, it was not possible to study the transport of all kinds of viruses in all kinds of soil materials. It is expected, however, that information gained about the transport and retention of albumin in the materials used will point the way to additional studies involving other proteinaceous particles and other soil materials.

The specific aims of this study were:

1. To study the transport and retention of proteinaceous particles by water in both fully and partially saturated sands with hydraulic gradients that occur in nature, and

2. To explain the mechanism by which such particles are retained in soil materials and to determine the variables affecting retention.

CHAPTER II

REVIEW OF LITERATURE

The material in this chapter falls under three headings: (1) Properties of Protein Particles, (2) Flow in Porous Media, and (3) Retention of Small Particles in Porous Media.

The material presented under the first two headings, although essential for an understanding of the research presented here, is not concerned with the specific objectives of the investigation. No attempt is made, therefore, to present a review of all the latest research on these subjects. Instead, a brief summary is made of the known information essential to an understanding of the phenomena investigated.

A. Properties of Protein Particles.

1. Physical -- The protein molecule is usually a long-chain structure of very high molecular weight (several thousand to several million) held together by peptide linkages, a form of covalent bonding. Proteins are, therefore, usually very complex molecules, some examples of these being the blood proteins, milk proteins, and egg proteins.

Viruses are proteinaceous particles of very small size, having a protein shell or envelope and containing large amounts of nucleoproteins within these envelopes. A partial list of some common protein particles has been compiled in Table 1, showing their approximate sizes and molecular weights. This information was obtained from Mysels (1959), Neurath and Bailey (1953), Adams (1959), and Pollard (1953).

TABLE 1

Proteinaceous Particles - Size and Shape

Name	Remarks	Size in Millimicrons	Molecular Weight x 10^{-3}
Bovine Serum Albumin	(ellipsoidal prolate)	4 x 16	69
Fibrinogen	cigar shaped	4 x 73	580
β_1 Lipoprotein	spherical	19	1300
Gamma Globulin		5 x 24	156
T-Series Coliphages	tails ≈ 25 x 10 m μ	65 x 95	
T-7 Coliphage	tails ≈ 10 x 15 m μ	47	
Tobacco Mosaic Virus	rodlike	15 x 280	40
Edestin	spherical	8	49
Insulin			12
Egg Albumin			34
Lansing Polio Virus		25	
Influenza Virus		150	
Foot and Mouth Disease		10	
Cocksackie, Texas		15 - 35	
Yellow Fever		22	
Catalase	small blocks	6 x 8	
Turnip Yellow Virus	spherical	20	
Human Serum Albumin	ellipsoidal	3.8 x 15	65.6 - 69.0

2. Chemical -- Proteins are constructed from α - amino acids linked by the condensation of the amino group of one acid with the carboxyl group of another forming the peptide linkage. In general, proteins are polypeptides and contain thousands of combinations (designated as R groups) linked together in chains which are usually folded. The chains may become stretched out, depending upon the presence of other proteins and salts in solution.

The R groups are specific for each amino acid. The folding or "packing" of the chain is influenced by the size and nature of the R groups, and the polarity of the R groups determines their affinity for other compounds. The characteristics of the R groups largely determines the physiochemical behavior of the proteins. There are four categories classified according to their charge and polarity:

a) non polar, as in glycine, alanine, and proline

b) cationic, as in lysine and arginine

c) anionic, present in aspartic acid

d) polar nonionic, as in serine and hydroxyproline.

Because the R groups contain both acidic and basic groups, the proteins are highly amphoteric; i.e., they can behave as acids or bases depending on the pH of the surrounding media.

In general, the proteins are easily changed, denaturation being the term used to describe such a change. Denaturation may be physical, chemical, or both. It can be brought about by changing pH, temperature, sound waves, surface forces, pressure, radiation, electrolytes and enzymes, among other things. A detailed discussion of all the physical and chemical properties that might be changed by denaturation is beyond the scope of this review. It is important, however, in working with protein particles to avoid procedures that lead to denaturation, some of which have been described by Anderson (1950), Adams (1948) and Francis, *et al.*, (1951).

3. *Electrokinetic and surface* -- The small size of the protein molecules and viruses greatly increases the relative importance of their surface properties. Most of the important physical, chemical and electrokinetic properties of proteins are due to their surface. Specifically, the electric charges of their surfaces in contact with solutions are important in adsorbtion as has been described by Alderton, *et al.*, (1945), and Kunitz (1946).

Every protein has an isoelectric point, i. e., a pH at which its average net charge is zero, and this has an affect on the extent to which it will adsorb on the surfaces of such materials as quartz (Abramson, 1932, and Dummett and Bowden, 1933).

B. Flow in Porous Media.

In 1931, L. A. Richards pointed out that Darcy's law can be used to describe flow in partially saturated as well as fully saturated soils. The coefficient of hydraulic conductivity in the case of partially saturated soils, however, is a function of the degree of saturation which in turn is a function of the liquid pressure.

Recently, Brooks and Corey (1964) developed a theory describing the interrelationship among saturation, pressure, and permeability of liquid and air. The fundamental considerations upon which the theory of Brooks and Corey is based are reviewed here because they lead to a better understanding of the way in which the transport of small particles might be affected by the degree of saturation. These considerations are as follows:

1. The mean length of the microscopic flow path of water when flowing through completely saturated porous media (which are isotropic) is about $\sqrt{2}$ times as great as the macroscopic flow path. This fact was discovered independently by several investigators whose work has been described by Wyllie and Spangler (1952).

2. The mean length of the microscopic flow path for isotropic media varies approximately as the inverse of the "effective saturation" of the porous media. This generalization was first discovered experimentally by Burdine (1953) and later was verified by Corey (1954). It was recently derived analytically by Wyllie and Gardner (1958).

3. The mean size and the maximum size of channels through which liquid flow takes place is reduced as the saturation is reduced. This fact can be deduced from elementary principles of surface physics and has been pointed out in numerous textbooks.

4. The maximum size of channels occupied by liquid at any particular saturation is related to the liquid pressure existing at that

saturation at equilibrium. This can also be deduced from elementary principles of surface physics.

5. The distribution of sizes of channels occupied by liquid at particular saturations can be related to curves of pressure as a function of saturation.

6. Brooks and Corey (1964) have shown that the relationship between pressure and saturation (over a large range of pressures on the drainage cycle) can be approximated by an equation of the form

$$\frac{S - S_r}{1 - S_r} = \left(\frac{P_b}{P_c}\right)^{\lambda} \quad \text{for } P_c \geq P_b \text{, and } S > S_r \tag{1}$$

in which S is the saturation expressed as a fraction of the total pore volume; S_r is the "residual" saturation at which the "capillary" or negative liquid pressure, P_c , becomes very large; and P_b and λ are positive constants, P_b being the minimum P_c at which a continuous gas phase exists throughout the porous medium. The expression on the left of equation 1 has been called the effective saturation S_e by Corey (1954).

The relationship between relative permeability and capillary pressure is

$$K_{ew} = \frac{K_{ew}}{K_w} = \left(\frac{P_b}{P_c}\right)^{\eta} \tag{2}$$

where $\eta = 2 + 3\lambda$, K_{ew} is effective permeability and K_w is saturated permeability. The hydraulic properties P_b , S_r , λ , and K_w are characteristics for a given media which must be determined in the laboratory (Brooks and Corey, 1964).

7. At any particular value of P_c, porous media tend to hold more water on the drainage than on the imbibition cycle.

C. Retention of Small Particles in Porous Media.

Very little information is available concerning the seepage of protein particles through soil under hydraulic gradients found in the field. Papers reviewed here deal mainly with retention of particles in filters used in water purification.

1. Retention of inorganic particles -- Suter (1956) employed a sand filter in an attempt to remove silt and clay particles from water used for ground-water recharge. He subsequently discovered that gravel, 4 - 5 mm. in diameter, was very effective and did not clog as did the filter sand. He noted that although some silt penetrated the gravel layer, most settled out on top, building up a layer 1 - 2 inches thick on the surface.

Ghosh (1958) studied the filtration of water made turbid with Fuller's earth. The filter columns were 3½ inches in diameter and 30 inches long. Some were filled with sand and some with small glass beads. He found that although the sand offered less resistance to flow, it retained more sediment than the glass. With fine-grained filters (in the presence of a coagulant), the top half-inch of the filter became heavily loaded but with the coarse-grained filters, the

Fuller's earth was well distributed throughout the bed. In the absence of coagulants, no particular layer removed more sediment than any other layer. The coarseness of the filter material had little effect on the amount finally removed.

Curry and Beasley (1962) measured the streaming potential across a column packed with carborundum particles and were able to show how the zeta potential of that medium decreased as clay particles of a bentonite slurry were removed from suspension and became trapped by the medium.

Rausch and Curry (1963) demonstrated that a decrease in zeta potential of a clay suspension resulted in increased removal of the clay particles from suspension by a carborundum porous media Curry, Barker and Strach (1964) concluded that the difference in negative zeta potentials between the clay and the carborundum probably controls the amount of retention of clay in the medium.

Jorden (1963) filtered clay suspensions through gravel filters at rates of 0.1 g.p.m. per ft^2, the filters having been initially operated for 65 days with local reservoir water to build up a "schmutzdecke" on the surface. The reduction in turbidity between influent and effluent solutions was found to correlate well with the electrophoretic mobility of the solutions. The author postulated that if removal were due to sieving or sedimentation, the larger particles would be preferentially removed. Measurements of the specific surfaces of the influent and effluent solutions showed that no consistent fractionation occurred.

The author concluded that removal was probably caused by adsorption of the clay particles in the "schmutzdecke."

Camp (1964) (in discussing the effect of floc deposition in the pore spaces on the Kozeny equation for flow through granular materials) reviewed some of the experiments of Stein* on the nature of clogging in rapid sand filters. Stein constructed a special two-dimensional filter element for mounting on a microscope so that he could observe the mechanism by which floc particles were removed from suspension in rapid sand filtration.

The grains in Stein's filter element did not touch one another so that removal could not occur as a result of sieving or sedimentation. An iron-manganese floc was specially prepared which was suitable for visual and photographic observation. The floc particles appeared to attach themselves to the grains forming whisker-like structures and at the higher flow velocities exhibited a slow creeping action along the grain surface. Initial contact was thought to be brought about "principally by the convergence of streamlines at contractions in the pore channels and in the vicinity of curved surfaces of the grains."

2. Retention of microorganisms (bacteria-sized) -- Krone, et al., (1958) studied the filtration of settled sewage and of solutions of pure bacterial culture through sandy soils. It was noted that there was a

---

*Stein, P. C., "A study of the theory of rapid filtration of water through sand." Unpublished Ph.D. Thesis, M.I.T.

sharp drop in bacteria count just below the surface, the highest count being close to, or at, the surface while further down in the column, the decrease in concentration was logarithmic. A mathematical relationship was derived, similar to that of Hall (1957), which predicted only the logarithmic portion of the distribution.

The mechanism of removal was postulated by the authors to be as follows: Initially both straining and sedimentation occur, and the straining mechanism increases the removal rate until accumulations in the contact zone become unstable; "... portions break off as clusters which are subsequently removed by straining or sedimentation. Accumulation probably occurs principally near the soil surface. When the first clusters break off at the surface, an avalanche of bacteria rapidly saturate the straining sites for some distance below, after which the saturated front moves downward more slowly." Supporting data were obtained by withdrawing samples of solution from the columns at various depths.

Heiple (1959) used $\frac{1}{4}$ - $\frac{1}{2}$ inch diameter washed river gravel at flow rates from 0.10 to 1.0 g.p.m./ft^2 through a 16-inch filter bed. This was not effective in removing algae but did remove fine suspended sediment and reduced the bacteria count about 60%. He found that filtration efficiency was greatest in low turbidity waters in which colloidal size particles predominated. Even after this filter had been in operation for 7 months, bacterial removal was as high as 70% and averaged 61% based on a membrane filter method of bacterial counting (McKinney, 1962).

Borchardt and O'Melia (1961) studied the removal of algae by rapid filtration through sand. All experiments showed the same general trends, namely, a decrease in removal efficiency and an increase in head loss with time. The mechanism of removal was not known, the experiments being restricted to studying the effects of removal.

Ives (1961) studied the distribution of radioactive algae as a function of depth and time in a sand filter operating at rates of 120 - 360 gal. per sq. ft. per hour. A theory of filter behavior was developed which is based on the following assumptions:

> "1. The removal of suspended material from water by a filter layer is proportional to the quantity of suspended material present in the water.
>
> 2. The removal characteristic of the filter depends on the surface available on the filter grains, on the tortuosity of flow within the pores, and on the interstitial velocity.
>
> 3. The principle force operating to remove suspended particles from the flow stream is gravitational, although adsorption forces become dominant when a particle reaches a grain surface."

From these assumptions, a differential equation was developed relating the changes in concentration as a function of depth to a parameter of the clean filter and the quantity of deposit in the pores. The equation was solved with a computer, and the solution was compared with results of an experiment operated under the conditions:

> "1. Uniform suspension remaining constant with time, flowing into the filter,
>
> 2. Suspension particles unisize and of definable geometry,

3. Uniform size of filter media, and

4. Constant temperature and constant flow rate."

The concentration of deposit was determined with a scintillation counter, the algae having been made radioactive with cesium-137.

The solution gave a relation among concentration of deposit, depth and time which compared qualitatively with the experimental results.

D. G. Zvyagintsev (1962) studied the behavior of microorganisms in soil solutions. The studies were made under a microscope using a suspension of soil and microorganisms in water. He found that clay soils are capable of adsorbing a large amount of varied types of microorganisms, and that in certain regions of the soil particle surface, the cells continuously adsorbed onto the soil, yet in other areas no apparent adsorption occurred.

3. Retention of virus-sized particles -- Carlson, et al., (1942) (using an adopted strain of poliomyelitis virus) studied the effects of standard purification methods on virus removal. Using a 30-inch Ottawa sand filter at a flow rate of 1.4 g.p.m. per ft^2, they found that sand filtration alone without alum treatment and floc formation was of little value. The use of activated charcoal instead of sand caused a substantial increase in virus removal by filtration.

Kempf, et al., (1942) studied the individual effects of flocculant, sedimentation, sand filtration and chlorination on polio virus. An Ottawa sand filter operating at 2 g.p.m. per ft^2 was able to remove 99.6% of the bacteria Escherichia Coli, but the viruses were not consistently removed even by a combination of alum treatment and filtration. Only by greatly increasing the alum concentration, were they able to remove all the viruses. Since the data were measured by a biological assay involving the infecting of monkeys, there is some doubt that 100% removal was obtained even then.

Samples taken from four water purification plants selected at random did not contain a high enough alum concentration to sediment the polio virus. The concentration of chlorine which would have been required to inactivate either of the strains of virus used in the experiments was in excess of that usually recommended for the production of bacteriologically safe drinking water.

Dieterich (1951) filtered solutions of a staphylococcus bacteriophage of micrococcus pyogenes through columns of Ottawa sand (40 - 50 mesh, U S. Sieve size), Columns pretreated with egg albumin showed no ability to remove the phages. Subsequent bottle tests using fresh sand and phages were used in an attempt to plot Freundlich and Langmuir adsorption isotherms. There was considerable scatter in the data which may have been caused by inaccuracies of the biological assay technique used. After considerable filtering had occurred, the ability of the sand to remove particles decreased.

Natural flow rates were used by Robeck, et al., (1962) in tests on attenuated poliovirus solutions passing through 2-foot beds of California dune sand. Based on biological assays of the effluent solution, it was found that the sand beds removed over 99% of the viruses for 98 days. When the flow rates were increased from

3 feet per day to 2 g.p.m. per ft^2, the removal rate decreased to about 10%. Contrary to the results of Dieterich (1951), they observed no decrease in the ability of the sand to remove particles even after 7 months of daily feeding of fresh viruses.

Using the coliform-bacteria membrane filter test as an index of viral concentration, Robeck, et al., (1964) compared the filtration efficiencies of coal, activated carbon and sand. A layer of coal on top of a sand filter considerably increased its effectiveness. Carbon previously exposed to large amounts of soluble organic contaminants was little better than plain sand in its ability to remove viruses.

D. Mathematical Analysis of Filtration.

Hall (1957) postulated that the reduction in concentration of suspended matter is continuous and that only a portion is removed at any given depth. He believed that the processes responsible for removal were chiefly straining and sedimentation. In the preliminary analysis, he assumed that two mechanisms carried material into a pore, namely, the flow of liquid carrying the solids in suspension and secondly, a slow creeping movement of the thick semi-solid material already deposited along the pore boundaries. Considering movement of material resulting from both mechanisms, he derived what he called the fundamental differential equation of filtration. This equation is a statement of conservation of mass for the system described,

$$\frac{\partial w}{\partial t} = -v \frac{\partial c}{\partial z} - \frac{\partial(\gamma_c v_c)}{\partial z}$$

where w $\equiv$ weight of sediment accumulated per unit of filter volume at any point - F/L^3 ,

v $\equiv$ rate of water flow through the filter - L/T,

c $\equiv$ concentration of suspended matter - F/L^3 ,

γ_c $\equiv$ specific weight of creep flow sediment - F/L^3 ,

v_c $\equiv$ creep flow rate - L/T,

z $\equiv$ depth - L.

Omitting the term for creep flow, and assuming that

$$\frac{dc}{dz} \approx \frac{\partial c}{\partial z} ,$$

which could be approximated at some instant of time by $-rc$, he obtained

$$w = v\,r\,c_o\,t\,e^{-rz} ,$$

where c_o is the initial concentration of suspended matter in the water entering the column, and r is an assumed factor of proportionality.

For interstitial straining, Hall suggested that r is a function of pore geometry and for sedimentation, a function of particle mass, diameter, and mean flow velocity. The equation was solved using a geometrically determined rate factor r , and the results showed good agreement with data published by the ASCE Committee on Sanitary Engineering, 1936, for the distribution of 10 - 25 micron sediment (as a function of depth) in a filter.

Bodziony and Litwiniszyn (1962) approached the problem from a more general point of view. Making no assumption concerning the physical mechanism by which particles are removed from suspension, they classified pores according to their particle retaining ability. They assumed that pores of a certain class may be characterized by the radius of the smallest particles capable of being retained in them and that particles smaller than this would not be retained by pores of that size.

Using a deterministic approach, they obtained a phenomenological equation describing the kinetics of the process which they combined with a continuity equation. The solution to the combined equation gave an exponential relationship for the number of pores filled with particles as a function of time and distance.

In a subsequent study, Litwiniszyn (1963) used a stochastic approach to the same phenomena and obtained an equation describing the kinetics of the process similar to that obtained earlier by the deterministic approach.

CHAPTER III

THEORY

Several possible mechanisms by which protein particles could be removed from suspension in flowing ground water can be postulated. The particles could be sieved out as a result of being caught in the crevices between adjacent soil particles. Another possibility is that sedimentation of the protein particles might occur on grain surfaces within the pores.

At certain positions within each pore, both gravitational forces and centrifugal forces operate on the protein particle causing it to move toward the soil surface. In a closed pore, simple sedimentation might occur in accordance with Stokes' Law. In an open pore, the additional centrifugal forces developed by the curvature of the flow around the soil particle might force the protein particle to settle out on the surface of the soil. Such removal mechanisms have been studied by several investigators in considering the flow of bacteria, clays, etc., in suspension, through a porous matrix. These mechanisms can be shown in some instances to result in an exponential distribution of the particles as a function of depth in the soil column. This apparently does not always explain the entire removal process, particularly near the upper surface of the permeameter (Curry, et al., 1964).

Another way in which the particles might be removed from suspension is by adsorption on the soil particles or at the gas-liquid interfaces formed when air is trapped within the porous media.

A. Analysis of Removal.

Regardless of the mechanism postulated for removal, the concentration of the disperse phase and the "trapped" phase should be expressible as some function of depth and time.

"Column kinetics" as this type of process is commonly called, does not lend itself readily to mathematical analysis. The literature on ion-exchange is replete with examples of semi-empirical and empirical solutions, and although the following approach is greatly simplified, it may be as valid as any other.

Assumptions:

1. The soil is homogeneous and isotropic.
2. The flow rate of water is constant.
3. The degree of saturation of the soil is constant.
4. The input of protein solution into the column is in the form of a "slug", i.e., a very thin wafer of the same area as the column but having a very small thickness compared to either the length or diameter of the column.
5. The concentration of protein is the same throughout the volume of the slug.
6. The velocity v_y of the protein particles in the slug is constant.
7. Diffusion effects are negligible.

A vertical column of soil is considered with the y axis parallel to the column and increasing in the downward direction. For very small amounts of protein introduced into the column, one can visualize the protein as a thin wafer of thickness w, moving downward through the column at a constant velocity v_y. Because of the very tortuous and interconnected nature of the channels, it is assumed that sufficient lateral mixing occurs to maintain a uniform concentration throughout the protein wafer. Since the wafer travels at constant velocity, the coordinate of the wafer is given by $y = v_y t$. Any protein lost by the wafer in traveling a distance Δy must equal the amount gained by a differential volume of soil equal to $\Delta y A$; i. e.,

$$(C_y - C_{y+\Delta y}) A w \phi S \rho = \theta A \rho_b \Delta y \qquad (1)$$

where

$C \equiv$ concentration of protein in solution (dimensionless)
$A \equiv$ cross-sectional area of column (L^2)
$w \equiv$ thickness of the wafer (L)
$\phi \equiv$ porosity of the soil matrix (dimensionless)
$S \equiv$ degree of liquid saturation (dimensionless)
$\rho \equiv$ density of water (M/L^3)
$\theta \equiv$ concentration of protein retained by the soil (dimensionless)
$\rho_b \equiv$ bulk density of the soil (M/L^3).

Dividing both sides of this equation by $\Delta y A$ and taking the limit as Δy approaches zero, one obtains

$$-\frac{dC}{dy} = \frac{\rho_b \theta}{\rho w \phi S} \qquad (2)$$

which is a material balance equation for the system described and does not impose any physical restrictions on the removal process.

1. Removal by sieving -- In soils and sands, the pore spaces are far from uniform, being highly tortuous, interwoven, and varying continuously in direction, size and shape. As the soil becomes desaturated, a greater proportion of the liquid flows through the smaller passages, and the filaments of liquid become even more tortuous. The radii of curvature of the liquid-air interfaces are closely related to the size of these filaments by the Young-LaPlace equation:

$$P_c = \sigma \left(\frac{1}{r_1} + \frac{1}{r_2}\right)$$

where r_1 and r_2 are the principal radii of curvature at any point on the surface, σ is the surface tension and P_c is the capillary pressure. For spherical surfaces this reduces to

$$P_c = \frac{2\sigma}{r} .$$

For non-spherical interfaces, such as those found in partially-saturated porous media, this relation may be expressed as

$$P_c = \frac{2\sigma}{r_e}$$

where r_e is an effective radius closely related to the average dimensions of the pore spaces occupied by water. It is sometimes called a mean curvature for non-spherical interfaces.

As the protein solution flows through a porous matrix, some of the protein particles flow through channels whose dimensions are large compared to the size of the particles. Others are in pore spaces in which $2r_e < d_p$, where d_p can be thought of as an effective particle diameter. These particles will be trapped or sieved and the variable θ in equation (1), in gms. of protein per gm. of soil, can be used to describe them. It is convenient to use increments of both time and space in analyzing the removal, and subsequently relating them through the expression for the velocity of the protein wafer. Considering, once again, the same differential element, any protein lost by the wafer is lost in these small pores and can be expressed in terms of the volumetric flow rate through small pores.

Assuming that all protein particles flowing through pores smaller than r_e in a time increment Δt are trapped in these pores over a distance Δy and that the concentration entering the small pores is the same as that in the large pores, one can write an expression for this removal process as:

$$Q' \rho (C_{y+\Delta y} - C_y)\Delta t = \rho_b A \Delta y (\theta_{t+\Delta t} - \theta_t) \ ,$$

where Q' is the volumetric flow rate through pores smaller than $2r_e$ and the other terms are the same as for equation (1). Q' (the volumetric flow rate) is equal to $q'A$ where q' is the flux through small pores expressed in dimensions of L/T . When this is substituted in the above equation, both sides of the equation divided by $A \Delta y \Delta t$ and the limit taken as $\begin{pmatrix} \Delta y \rightarrow 0 \\ \Delta t \rightarrow 0 \end{pmatrix}$, the result is

$$q'\rho \frac{\partial C}{\partial y} = \rho_b \frac{\partial \theta}{\partial t} \quad .$$

In the system described, θ is a function of concentration C and time, and C is a function of time. Since $y = v_y t$, the time and space coordinates are isomorphic. Thus,

$$\frac{\partial \theta}{\partial t} = \frac{d\theta}{dt} = \frac{d\theta}{dy}\frac{dy}{dt} = v_y \frac{d\theta}{dy} \; ; \; \frac{\partial c}{\partial y} = \frac{dc}{dy} \quad .$$

and the equation describing the removal is

$$q'\rho \frac{dC}{dy} = \rho_b v_y \frac{d\theta}{dy} \quad . \tag{3}$$

Substituting equation (2) into equation (3) and dividing both sides by ρ_b , results in

$$- \frac{q'}{v_y} \frac{\theta}{w\phi S} = \frac{d\theta}{dy} \quad . \tag{4}$$

Rearranging terms, an equation is obtained which is integrable in θ and y ; i. e.,

$$\frac{-q'}{v_y w\phi S} \int dy = \int \frac{d\theta}{\theta} \quad .$$

Integrating and utilizing the boundary condition $\theta(o) = \theta_o$ results in the following expression for θ as a function of the depth y :

$$\theta = \theta_o \, e^{-\left(\frac{q'y}{w\phi S v_y}\right)} \tag{5}$$

The volume flux q' (through the small pores) can be related to the permeability by the Darcy equation:

$$q' = \frac{\rho g K'_{ew}}{\mu} \frac{d\left(\frac{P^*}{\rho g}\right)}{dy} \tag{6}$$

where g = acceleration of gravity (L/T^2)

K'_{ew} = effective permeability of the small pores (L^2)

μ $\equiv$ dynamic viscosity (M/LT),

P^* $\equiv$ piezometric pressure (M/LT^2).

Other variables are the same as previously noted, and the effective permeability K'_{ew} equals the product of the saturated permeability K_w and the relative permeability K'_{rw} ; i. e.,

$$K'_{ew} = K'_{rw} K_w \quad . \tag{7}$$

Ordinarily, K_{ew} is defined as the permeability of a porous medium to a particular fluid phase when the medium is occupied by more than one fluid phase and K_w is defined as the maximum permeability to a particular fluid phase when only one phase occupies the media. The latter is a function of the geometry of the media and not of the fluid properties. The sense in which the primed symbol K'_{ew} in the above equation is used is different. It represents the permeability of the small pores $(2 r_e < d_p)$ of a porous medium to a particular fluid phase when the medium is occupied by more than one fluid phase.

It can be shown (Brooks and Corey, 1964) that the effective permeability can be represented as follows:

$$K_{ew} \approx \frac{\phi S \overline{R_w^2}}{k T} \tag{8}$$

where $\overline{R_w^2}$ is the mean of the square of the hydraulic radius, k is a shape factor and T is the tortuosity. In equation (8), it is reasonable to assume that ϕ and k are independent of the saturation. The ratio $K_{rw} = \frac{K_{ew}}{K_w}$ can be written as

$$K_{rw} \approx S \frac{T_{1.0}}{T_S} \frac{[\overline{R_w^2}]_S}{[\overline{R_w^2}]_{1.0}} \quad . \tag{9}$$

The subscripts 1.0 and S refer to the variable at a saturation of 1.0 and at any particular saturation S respectively. An expression can be obtained for $\overline{R_w^2}$ as a function of saturation; namely,

$$\overline{R_w^2} = \frac{\sigma^2 \cos^2 \zeta}{S} \int \frac{dS}{P_c^2} \tag{10}$$

where σ is the surface tension, and ζ is the angle of contact of the wetting phase at the solid interface (Wyllie and Spangler, 1952).

Burdine (1953) showed that the ratio of tortuosities could be empirically expressed as

$$\frac{T_{1.0}}{T_S} \approx S_e^2 = \left(\frac{S - S_r}{1 - S_r}\right)^2 \quad , \tag{11}$$

which when substituted into equation (9) together with equation (10), leads to

$$K_{rw} \approx S_e^2 \frac{\int_o^S \frac{dS}{P_c^2}}{\int_o^1 \frac{dS}{P_c^2}} \tag{12}$$

where S_r is called the residual saturation, a saturation at which K_{ew} is assumed to be zero.

Brooks and Corey (1964) demonstrated that S and P_c are empirically related by

$$S_e \approx \left(\frac{P_b}{P_c}\right)^\lambda \tag{13}$$

where λ is called the pore-size distribution index, and P_b is called bubbling pressure. The bubbling pressure is the minimum capillary pressure on the drainage cycle at which a continuous air phase exists in the media; hence, it is a measure of the largest pores present in the soil matrix. The pore-size distribution index λ is a measure of the uniformity of the pores, a small value of λ indicating the percentage of pore space to be rather evenly distributed among the different pore sizes, whereas a large value of λ indicates that most of the pore volume is within a narrow pore-size range.

It is expected that $\overline{R_w^2}$, the mean of the square of the hydraulic radius, would be quite different for pores smaller than r_e when compared with all the pores. Equation (10), which expresses $\overline{R_w^2}$ as a function of the degree of saturation, should be applicable to these pores if the integral is taken from S_r to the saturation corresponding to the radius r_e , i.e., when $P_c = \frac{2\sigma}{r_e}$.

The tortuosity is a measure of the ratio of the distance between two points in a porous matrix (parallel to the direction of flow) to the actual length of the tortuous passage between the two points. For a given degree of saturation, it seems reasonable to assume that the tortuosity of the flow paths would be the same for large pore spaces as for small pore spaces. Physically, this might be interpreted to mean that a fluid element would have to travel the same distance to go from y_1 to y_2 regardless of whether it traveled through small pores or large ones.

Utilizing these assumptions, an expression for K'_{ew} analogous to equation (8) is obtained which includes the effect of saturation but refers specifically to the permeability of pores whose effective radius is less than r_e :

$$K'_{ew} = \frac{\phi\, S(r_e)\, \overline{R_w^2}\,(r_e)}{k\ T(S)} \quad . \tag{14}$$

The symbols (r_e) and (S) indicate that the variables preceeding them are functionally dependent upon them and do not symbolize scalar multiplication of the quantities. Accordingly, one can write

$$\overline{R_w^2}\,(r_e) = \frac{\sigma^2 \cos^2 \zeta}{S(r_e)} \int \frac{dS(r_e)}{P_c^2(r_e)} \ , \tag{15}$$

and

$$K'_{rw} = S_e^2\,(S)\, \frac{\displaystyle\int_o^{S(r_e)} \frac{dS(r_e)}{P^2\,(r_e)}}{\displaystyle\int_o^{1} \frac{dS(S)}{P_c^2\,(S)}} \quad . \tag{16}$$

This means that S_e^2 is dependent upon the degree of saturation and that integration of the denominator must proceed from $S = 0$ to $S = 1.0$, whereas the integral in the numerator is taken from $S = 0$ to an S corresponding to $P'_c = \frac{2\sigma}{r_e}$.

By making a change of variable from S to S_e and expressing P_c^2 in terms of S_e by equation (13), this equation can be integrated to yield

$$K'_{rw} = S_e^2\,(S)\, S_e^{\frac{2+\lambda}{\lambda}}\,(r_e) \ , \tag{17}$$

and substituting equation (13) gives

$$K'_{rw} = \left(\frac{P_b}{P_c}\right)^{2\lambda} \left(\frac{P_b}{P'_c}\right)^{2+\lambda} \tag{18}$$

where $P'_c = \frac{2\sigma}{r_e}$.

It is assumed that the velocity of the protein wafer will be proportional to the mean velocity of the fluid elements so that

$$v_y = \psi \bar{u}$$

where $\psi \leq 1.0$ and $\bar{u}$ is the mean velocity for fluid elements.

Since the volume flux q in a porous medium is related to the velocity $\bar{u}$ by $q = \bar{u} \phi S$, equation (5) becomes

$$\theta = \theta_o e^{-\left[\frac{q' y}{w \psi q}\right]} . \qquad (19)$$

For two-phase flow through porous materials, Brooks and Corey (1964) have shown that

$$K_{rw} = \left(\frac{P_b}{P_c}\right)^{2+3\lambda}$$

and therefore

$$q = \frac{\rho g}{\mu} \left(\frac{P_b}{P_c}\right)^{2+3\lambda} K_w \frac{d\left(\frac{P^*}{\rho g}\right)}{dy} .$$

If $K'_{rw} = \frac{K'_{ew}}{K_w}$ is substituted from equation (18) into equation (6) and this, together with the above expression for q , is substituted into equation (19), the result is

$$\theta = \theta_o e^{-\left[\frac{y}{w\psi}\left(\frac{P_c}{P'_c}\right)^{2+\lambda}\right]} . \qquad (20)$$

It must be recognized that θ_o in the above equation is not a constant but is very much dependent upon hydraulic conditions in the porous matrix. If equations (2) and (3) are combined and integrated in terms of C rather than θ and the result is expressed in terms of θ , this gives

$$\theta = \frac{\phi S \rho C_o}{\rho_b \psi} \left(\frac{P_c}{P'_c} \right)^{2+\lambda} e^{-\left[\frac{y}{w\psi} \left(\frac{P_c}{P'_c} \right)^{2+\lambda} \right]} \tag{21}$$

which indicates the nature of the parameter θ_o . The variable C_o represents the concentration of the wafer when $y = y_o$.

For fully saturated flow (i. e., for $P_c \leq P_b$) P_b should be used in place of P_c since it represents the largest pores so that

$$\theta = \frac{\phi S \rho C_o}{\rho_b \psi} \left(\frac{P_b}{P'_c} \right)^{2+\lambda} e^{-\left[\frac{y}{w\psi} \left(\frac{P_b}{P'_c} \right)^{2+\lambda} \right]} . \tag{22}$$

Clearly, if $P_b \geq P'_c$, all the particles will be trapped and equation (22) no longer applies. Equations (20) and (21) describe the distribution of protein retained by the column as a function of depth, the thickness of the wafer and the pertinent hydraulic properties of the soil and the water. It would be difficult to characterize an effective radius in terms of an effective particle diameter, so that the variable P'_c is still somewhat vague.

2. Removal by adsorption -- Another possible mechanism of protein retention in porous media is adsorption of protein particles on the surface of the media solids. Two types of adsorption are referred to in the literature, namely, "physical" adsorption and "chemisorption," although the ultimate distinction between the two has not been clearly stated.

For the purpose of this discussion, the term "physical" is applied to adsorption resulting from various electrostatic forces not including those involved in covalent or coordinate-covalent bonding. The term "chemisorption" is applied to reactions involving the latter types of bonding.

Chemisorption is believed to be unlikely when the porous media is sand and the protein particles are suspended in a practically neutral solution.

Several adsorption models have been devised, the applicability of any particular model being dependent upon:

(1) the physical and chemical nature of adsorbed particles and the material upon which they are adsorbed,

(2) the pH of the solution in which the particles are suspended, and

(3) the characteristics of the flow of the solution in which the particles are suspended.

The adsorption models describe the equilibrium value of the adsorbed phase in terms of the equilibrium value of the dispersed phase (concentration) at a particular temperature. A curve of the concentration of adsorbed phase θ as a function of concentration of dispersed phase C is called an adsorption isotherm. Some well-known types of adsorption isotherms are:

(1) $$\theta = kC \tag{23}$$

where k is a proportionality constant, de Boer (1953).

(2) $$\theta = aC^{1/n} \tag{24}$$

where "a" and "n" are empirical constants, equation (24) being known as the Freundlich isotherm (Adamson, 1964). In this equation "a" is a measure of the surface area of the solid media and "n" is related to the intensity of adsorption.

(3) $$\theta = \frac{abC}{1+bC} \qquad (25)$$

in which "a" is the ratio of the specific surface of the soil to the specific surface of the protein molecules and "b" is a function of the heat of adsorption.

(4) $$\theta = De^{kC} \qquad (26)$$

in which D and k are empirical constants. This equation represents a portion of a Brunauer, Emmett, and Teller isotherm (Adamson, 1964).

Most of these models were derived empirically from observations of various kinds of adsorption phenomena. Equation (25), the Langmuir equation, was derived from kinetic theory for the case of gas adsorption from solutions. It applies rigorously only for the case in which adsorption occurs in a mono-molecular layer on the adsorbing medium.

In attempting to discover which of these models, if any, best describes the retention of protein particles in porous media, the following conditions are assumed:

(1) The media initially contains no adsorbed protein.

(2) The particles are carried into the media by the flowing solution with the same average velocity as the fluid elements.

(3) Dispersion of the particles by diffusion is negligible.

(4) The velocity of the flow is sufficiently small that there is time for the adsorbed and dispersed phases of the protein

particles to reach equilibrium concentrations; i.e., an adsorption isotherm is applicable.

(5) As in the case of the sieving analysis, the protein suspension is added at the top of the column of porous media as a discrete increment, so that the protein suspension passes into and through the media in the form of a thin wafer of constant thickness. The thickness is very small compared to either the length or diameter of the column. The concentration of protein particles in the wafer decreases as it passes through the column because some of the particles are retained by the media.

It is expected that among the important variables involved are the rate of adsorption and the distance from the surface over which the adsorptive forces act in removing a particle from solution. The length of the tortuous path and the mean radii of the fluid filaments also should be important factors. It is probable that the distribution of pore sizes as measured by the index λ also has an effect.

Assuming that an adsorption isotherm can be found which describes the relationship between the adsorbed and dispersed concentration of protein particles, an expression for concentration of adsorbed protein θ as a function of depth below the surface y can be obtained as follows:

Both sides of the isotherm equation are differentiated with respect to y. The resulting expression for $\frac{dC}{dy}$ is then substituted into the material balance equation (equation (2)). The resulting differential equation is integrated to give θ as a function of y.

When this procedure is applied to the standard adsorption isotherms, the results are:

(1) For the linear isotherm,

$$\theta = \theta_o e^{-mky} \tag{27}$$

where θ_o is the value of θ associated with $C = C_o$, and $m = \frac{\rho_b}{w \phi S \rho}$.

(2) For the Freundlich isotherm,

$$\theta = \frac{\beta}{y^{\alpha}} + B \tag{28}$$

where $\beta = \left[\frac{1-n}{n} a^n m\right]^{\frac{1}{n-1}}$, $\alpha = \frac{1}{n-1}$, and B is a constant which must be evaluated at the lower end of the column for $n > 1$ and equals θ_o for $n < 1$.

(3) For the Langmuir isotherm,

$$y = \frac{1}{mb(\theta - a)} + \frac{1}{abm} \ln G \frac{(\theta - a)}{\theta} \tag{29}$$

where G is a constant of integration. It is difficult to determine the functional relationship $\theta = f(y)$ but by some approximations, it can be shown that this curve is concave upwards, asymptotic to both the θ and y axes and is roughly hyperbolic in form.

(4) For the isotherm of equation (26),

$$\theta = \frac{1}{mky + \frac{1}{\theta_o}} . \tag{30}$$

A more complete analysis should include the rate of adsorption, the effect of tortuosity, saturation, P_b, and λ, and the use of thicker increments of protein suspensions for which θ and C would vary both with time and distance. Such an analysis may become feasible when more is known concerning the adsorption mechanism. It should be noted that the analysis described above makes no assumption concerning the adsorption mechanism other than its being describable by an isotherm.

CHAPTER IV

PROCEDURE AND EXPERIMENTAL EQUIPMENT

A variety of sandy soils, containing little or no clay, were used in this study. The soils were packed in specially constructed plastic columns called permeameters, which facilitated control of the degree of liquid saturation, the hydraulic gradient and the capillary pressure during steady downward flow of water (or of an aqueous suspension of protein particles). The hydraulic conditions in the column were held constant while radioactively labeled albumin molecules were introduced at the top of the column as a discrete charge or "wafer."

During the passage of the protein solution, the column was scanned with a scintillation probe mounted outside the column and arranged so that it could be moved vertically along the axis of the column. This technique provided a qualitative estimate of the distribution of albumin in space and time. Clear water was permitted to flow through the column before, during and after the passage of the albumin charge. This was continued until the effluent from the permeameter showed no traces of radioactivity; whereupon the column was dismantled and sectioned, and the distribution of retained albumin was determined quantitatively using a well-type scintillation detector.

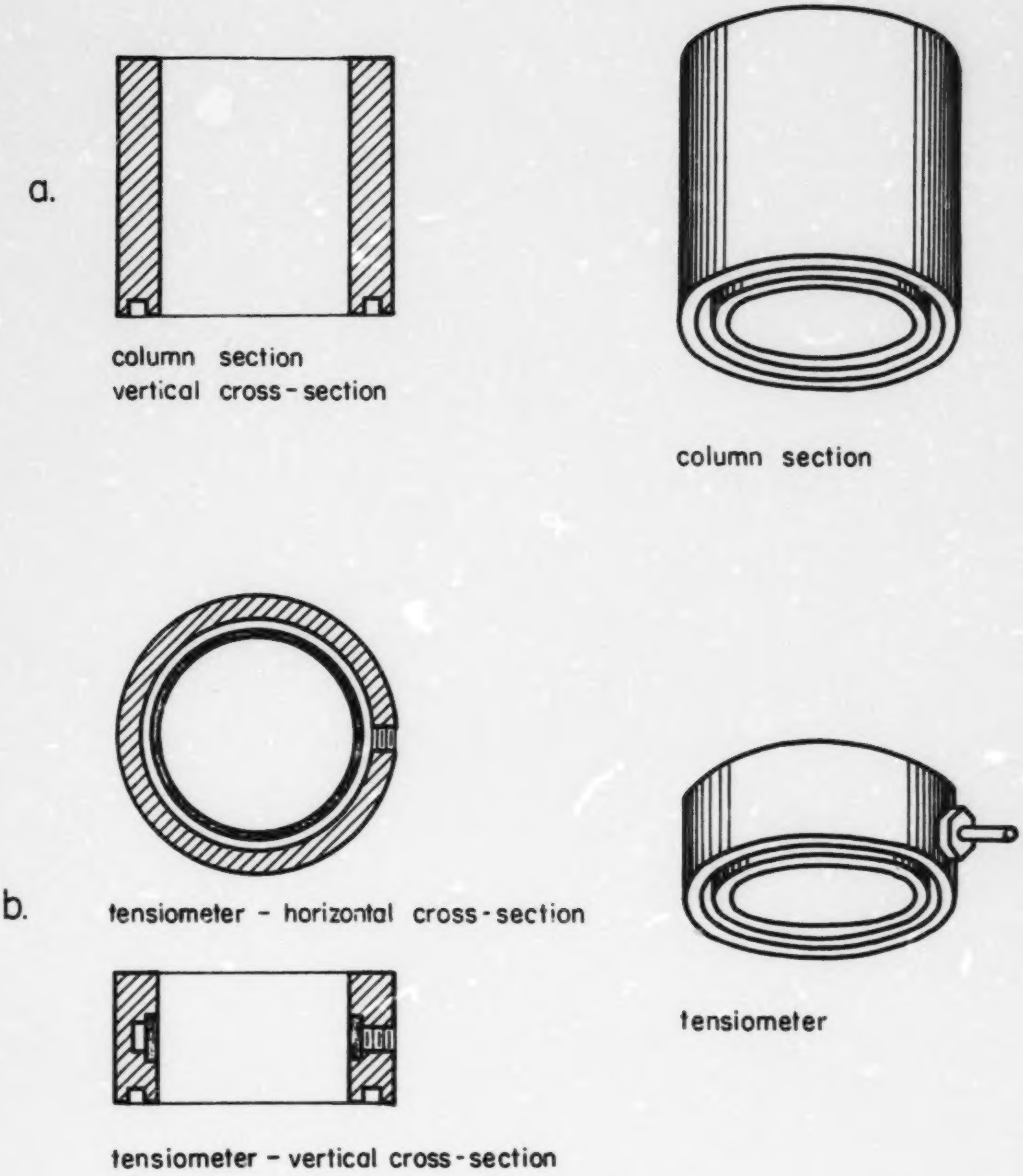
By maintaining a hydraulic gradient of unity, the hydraulic conditions selected for a particular run could be held constant throughout the permeameter. The hydraulic properties of the soils were determined in separate experiments using similar equipment. A series of separate experiments was also conducted to determine the specific surface of the materials and their capacity to adsorb protein particles. A modification of the procedure described above was used to determine conclusively whether or not the mechanism of removal was sieving.

The equipment used for studying protein particle transport was essentially the same as that employed by Brooks and Corey (1964) for determining the hydraulic properties (permeability, bubbling pressure, and pore-size distribution index) of soil materials.

Details of the experimental procedure and equipment are described below.

A. Hydraulic Equipment.

1. Permeameter sections -- The permeameters used to contain the soil consisted mainly of sections of acrylic tubing 4 cm. in length and 3.20 cm. inside diameter, Figure 2a. Shorter sections, 2 cm. long, called tensiometers (Figure 2b) were used for measuring the capillary pressure of the porous media. A detailed sketch of the tensiometers are shown in Figure 3. These were constructed by machining an annular groove about $\frac{1}{2}$ cm. wide around the inside wall of the plastic tubing. A second groove slightly deeper, but not as wide, was machined to permit the circulation of water around the circumference of the tensiometer. A thin circular section of porous ceramic



column section
vertical cross-section

column section

tensiometer - horizontal cross-section

tensiometer

tensiometer - vertical cross-section

Figure 2. Acrylic components of permeameters

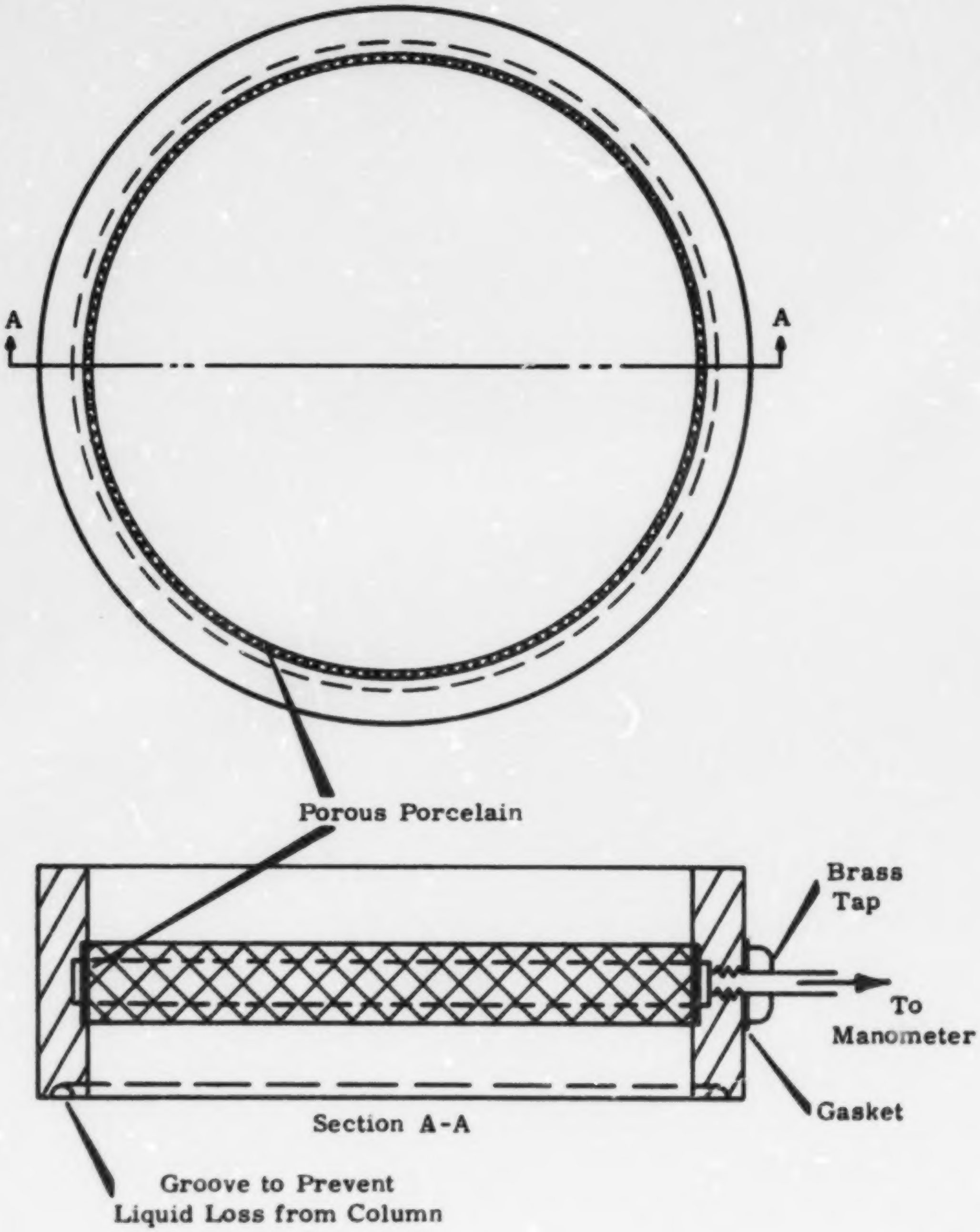

A
A
Porous Porcelain
Brass
Tap
To
Manometer
Gasket
Section A-A
Groove to Prevent
Liquid Loss from Column

Figure 3. Tensiometers used for measuring capillary pressure (Courtesy L. G. King)

material* was cemented** into the first groove to cover the deeper groove. This ceramic material was mounted flush with the inside of the column walls. A brass fitting was screwed into a threaded hole to connect the annular groove to a length of flexible plastic tubing leading to a manometer.

The outflow barrier was similar to the tensiometers, except that this section was 4 cm. long and closed at one end to keep the soil from falling out the bottom of the permeameter. The porous ceramic ring extended nearly the entire length of the section to provide a large cross-sectional area for flow. Tensiometers and capillary barriers were necessary because most of the flow occurred at water pressures less than atmospheric; and if the porous barriers had not been used, air would have entered the manometer and flow tubing. In some experiments, an outflow barrier consisting of several graded layers of sand was used in place of the ceramic barrier since albumin tended to plug the ceramic quickly.

As Figure 2 shows, the lower end of each acrylic section was grooved. This was to minimize flow of water out of the columns (at pressures near atmospheric) due to capillary "suction" at the joints.

---

*Selas microporous porcelain filter media, #015 porosity, obtainable from Selas Corporation, Dresher, Pennsylvania.

**Epoxy Resin, Armstrong's Adhesive Type A-1, obtained from Armstrong Products Company, Warsaw, Indiana.

2. Assembling the permeameters -- The permeameters were assembled by butting the plastic sections (including the tensiometers) together and holding them in place with strips of plastic tape* running the length of the column. The permeameters were usually about 30 cm. long with tensiometers spaced 10 cm. apart (see Figure 4).

Inflow of water, or protein solution, to the permeameter was accomplished by means of a controlled drip system (to be described) or an inflow barrier, shown in Figure 5. The porcelain disc (Figure 5) was of the same ceramic material previously described and was similarly cemented to the plastic with epoxy resin. This was held tightly against the soil by a spring shown in Figure 5 and was connected to a Mariotte siphon to maintain the liquid supply to the permeameter at a constant pressure and hence a constant flow rate. In some cases, a section similar to an outflow barrier (but open at both ends) was used at the top of the permeameter in place of the inflow barrier described above.

3. Packing -- The columns were packed with soil materials to a uniform bulk density utilizing a mechanical packer similar to one built by Jackson, et al., (1962) which has a motorized tremie that lays down a helical layer of soil in the column which is simultaneously vibrated by the vibrating block to which it is clamped. It was later

---

*Scotch Brand, vinyl plastic electrical tape no. 33, obtainable from Minnesota Mining and Mfg. Company, was found to be resistant to prolonged soaking in water.

Reproduced from
best available copy.

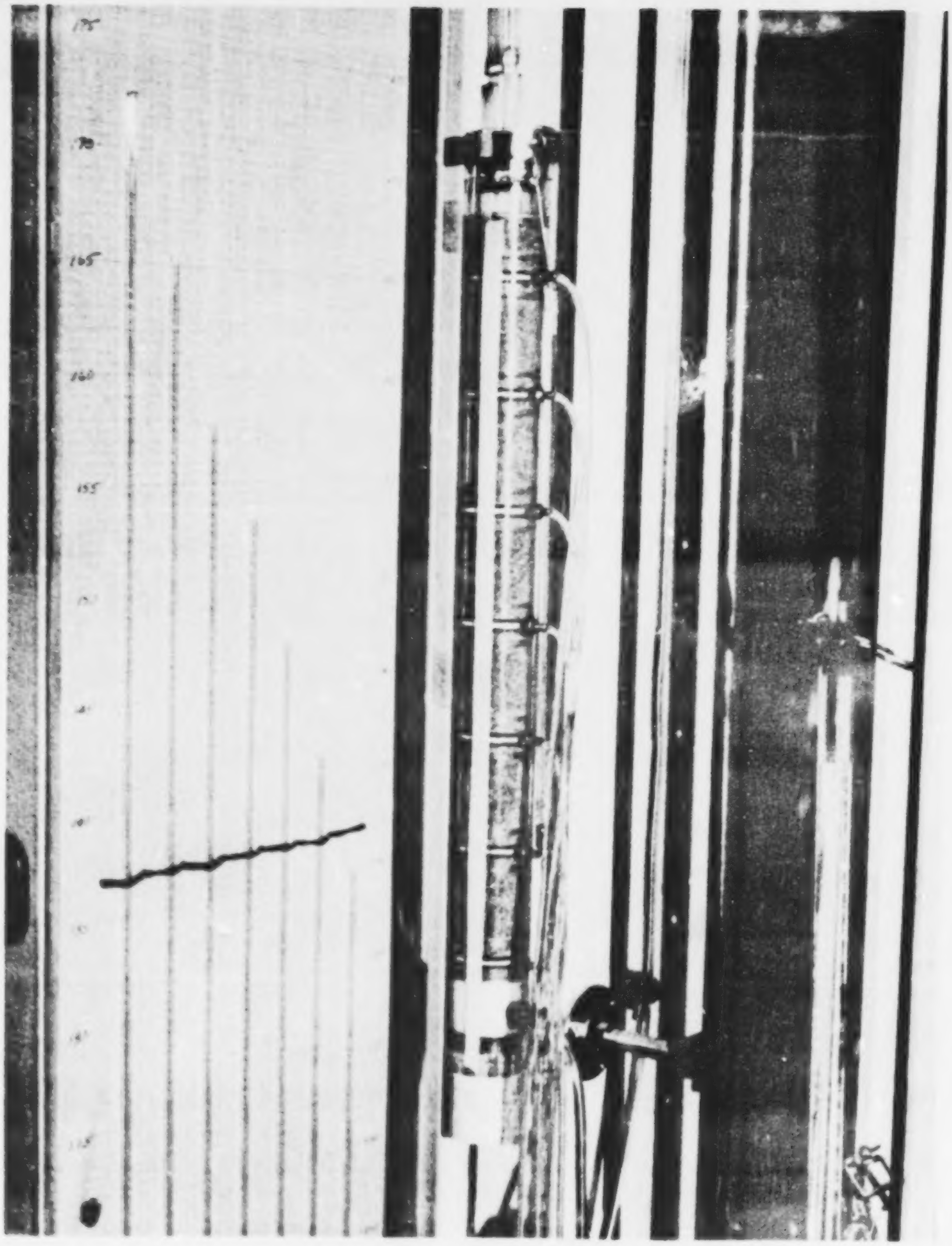

175
170
165
160
155

Figure 4. Assembled permeameter (Courtesy L. G. King)

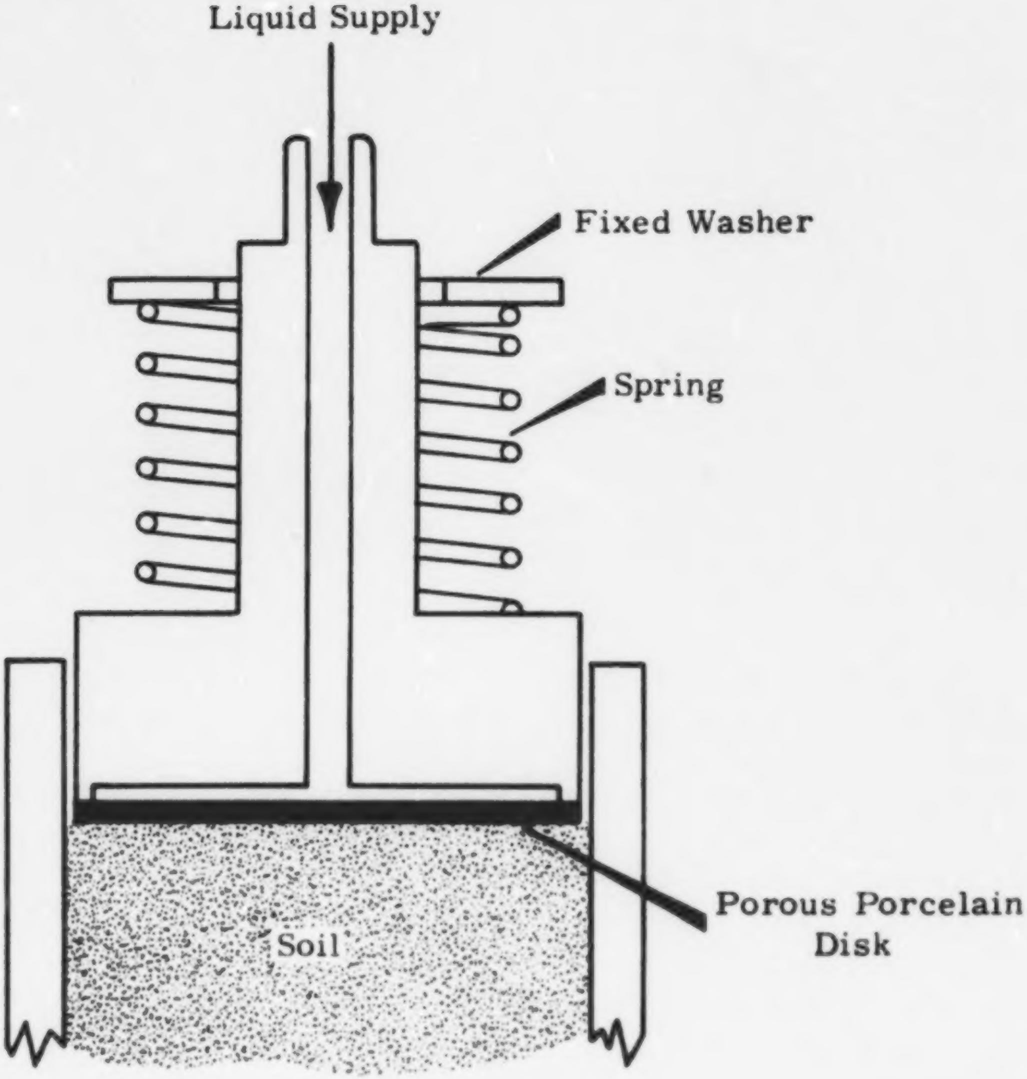

Liquid Supply
Fixed Washer
Spring
Porous Porcelain Disk
Soil

Figure 5. Inflow barrier assembly (Courtesy L. G. King)

observed that this type of packing resulted in considerable particle segregation and that better results could be obtained by vibrating after the column was filled rather than during filling.

4. Saturation of the soil -- All columns were vacuum saturated prior to making an experimental run. This process removed all air from the pore spaces and allowed them to be completely filled with liquid. The column as packed above was covered with a small circle of fiberglass gauze at the top which just fitted the inside diameter of the tube. This was held in place with a perforated plastic plug. The entire column was then clamped axially with a special clamp resembling a long, narrow U-bolt. This was to prevent the column from being elongated due to the action of expanding air bubbles. All manometer and barrier taps were fitted with short lengths of flexible plastic tubing. The permeameter was then lowered into a container of water or protein solution, and the container was sealed and placed under vacuum for about 30 minutes. In addition to removing the air from the pores of the soil, this also removed the air from the tensiometers, barriers and manometer leads. At this time, the permeameter was gradually brought back to atmospheric pressure and kept submerged for a few more minutes so that any air still present would have an opportunity to go into solution.

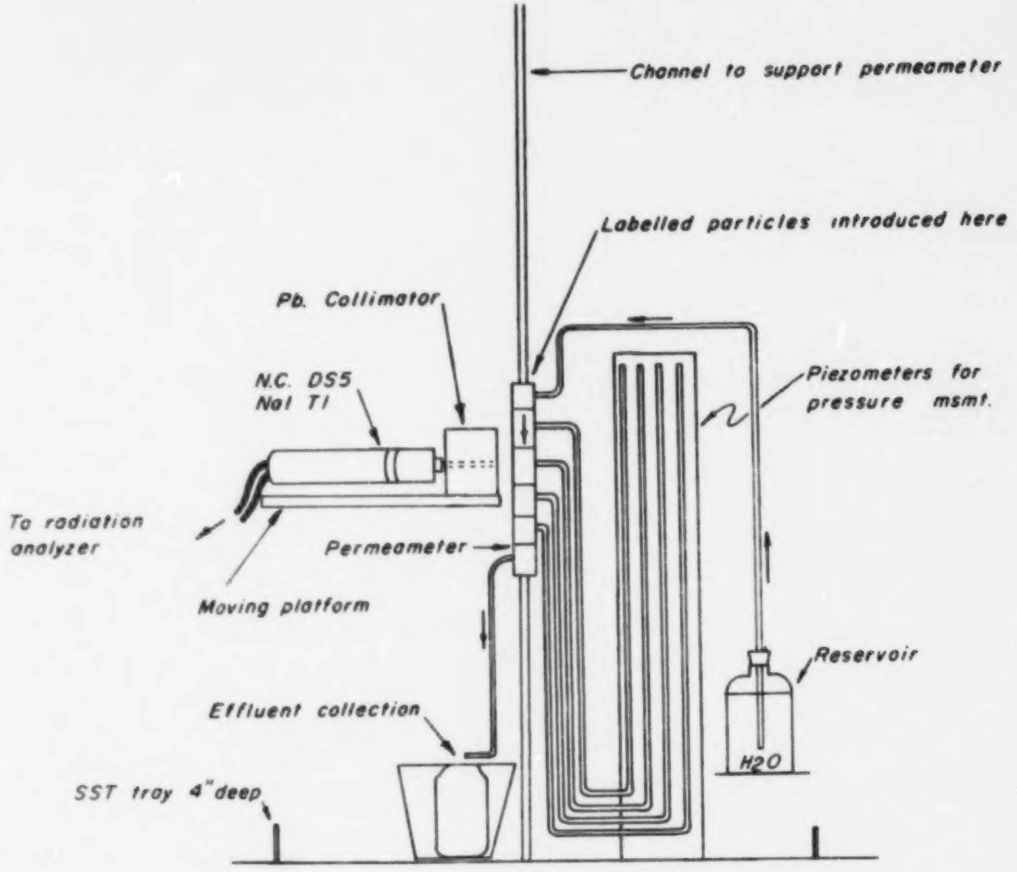
5. Setting up for the run -- If an inflow barrier was to be used, this was also vacuum saturated and connected to the Mariotte Siphon.

As the permeameter was removed from the container, the manometer and outflow leads were quickly clamped to prevent loss of water or entrance of air into the tensiometers. The column was then taped to a vertical length of channel iron opposite the collimator as shown in Figure 6. The inflow barrier was then lowered into place and the liquid allowed to flow. Care was necessary during this time to prevent the upper part of the column from desaturating since water was free to flow through all the joints between acrylic sections. Once the inflow barrier was connected, there was no difficulty in keeping the soil saturated. Next, the manometer leads were connected and then the outflow barrier was connected to an outflow siphon (plastic tubing). This was placed at some convenient elevation close to that of the outflow barrier.

6. Establishing hydraulic equilibrium -- Following the procedure for measuring relative permeability as a function of capillary pressure (see Brooks and Corey, 1964), the columns were brought to some predetermined capillary pressure over the entire length of the test section. This automatically controlled the degree of saturation throughout the test section and determined the flow rate at which the column would operate.

B. Materials Used in Transport Studies.

1. Media -- The following media were used in protein transport experiments:


Channel to support permeameter
Labelled particles introduced here
Pb. Collimator
N.C. DS5
NaI Tl
Piezometers for
pressure msmt.
To radiation
analyzer
Permeameter
Moving platform
Reservoir
Effluent collection
H2O
SST tray 4" deep

not to scale

Figure 6. Apparatus for studying the transport of radioactive protein particles

White sand - This is a very uniform water-deposited quartz sand containing no clay or silt. A sieve analysis is presented in Figure 7.

G. E. #8 sand - A fine sand with many different mineral constituents and slight amounts of clay and silt present.

Greeley sand - This is an extremely sandy agricultural soil, having a particle density of 2.68 gms. per cm^3 and containing almost no clay. The material used in the experiments consisted of fractions passing a 10 mesh sieve. An analysis is shown in Figure 7.

By utilizing these relatively coarse-grained materials, it was assumed that under saturated conditions, little or no sieving would occur and that by gradually desaturating the column (by increasing P_c), a saturation would be reached at which sieving would begin. Negligible adsorption was predicted since these materials had insignificant surface areas compared to agricultural soils, and at neutral pH used in the experiments, both albumin and soil particles would have a negative zeta potential and were expected to repel each other.

All the materials described above were heated to 1400°F in a muffle oven to destroy organic matter and any microorganisms which might have been present.

2. Selection of a virus-sized particle -- A particle was required which was in the size range of the viruses, readily available, and which would exhibit characteristics similar to those of a virus particle. Since no two viruses are the same, one would expect variations in

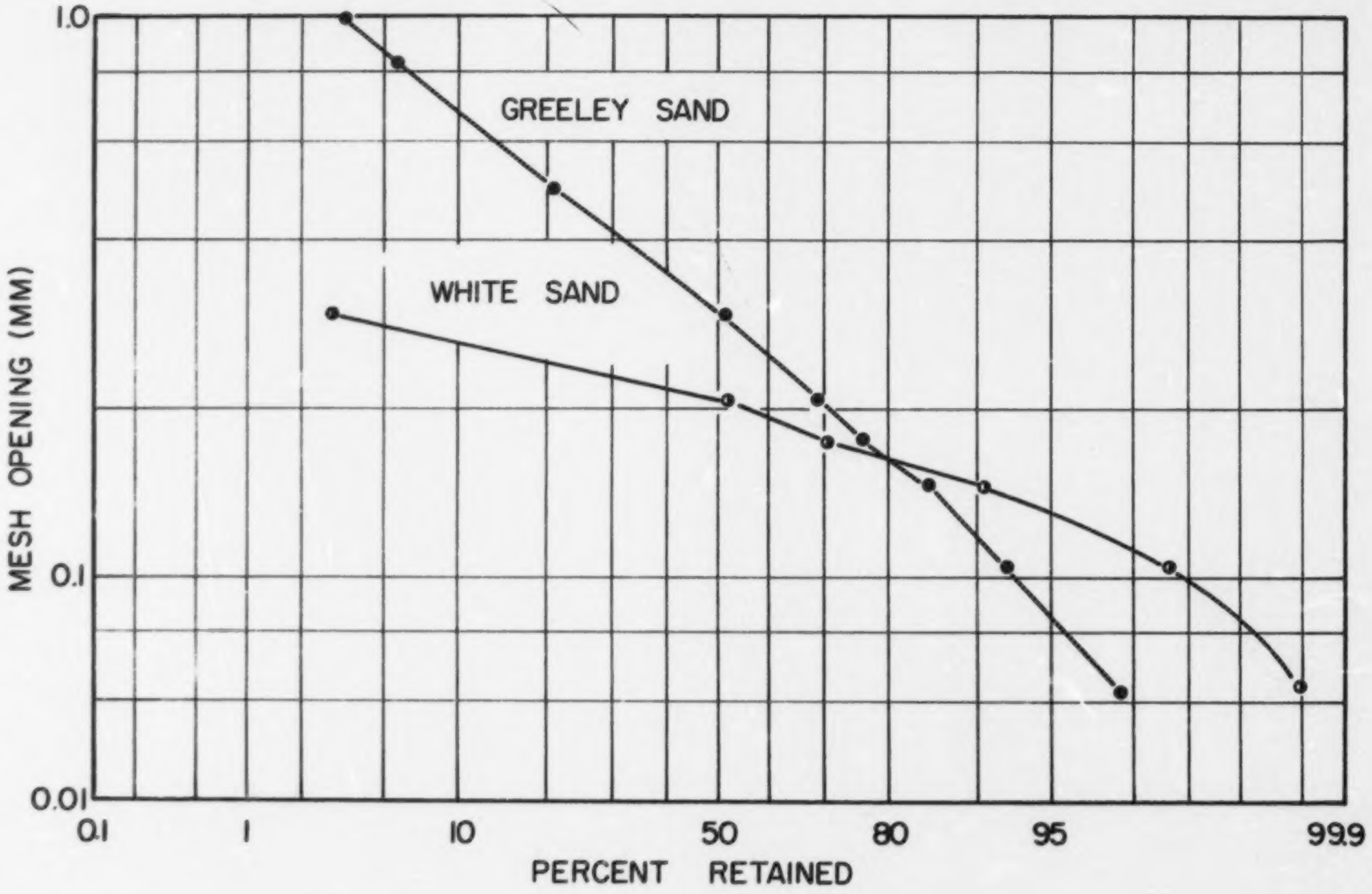


Figure 7. Sieve Analyses for Greeley sand and White sand

the behavior of different viruses. An "ideal" particle was needed which would more or less be a model for all ultra-small protein particles and would make possible some reasonable projections of experimental results.

Initially, the use of carbowax molecules was considered. These are highly polymerized molecules which have a repeating "R" structure resembling ethylene-glycol. Although they fall in the required size range, they have an odd shape which might have made their movement unpredictable. They range in length from 8 to 125 millimicrons and have a helical cross-section about 2 millimicrons in diameter and a molecular weight of 1,300 - 20,000.

Dyes, latex particles, talc and attenuated polioviruses were also considered. It was finally decided in light of the work of Chang, et al., (1958), that a bacterial virus, labeled with a radioactive tracer, probably would behave in a manner quite typical of viruses. The T-7 Coliphage was successfully labeled with Iodine-131, details of the procedure appearing in Appendix A. The equipment used for labeling the T-7 Bacteriophages and for working with the radioactive tracers was standard equipment normally found in a radiochemistry laboratory. This consisted of micropipettes and pipette controllers, tongs for handling bottles and tubes, laboratory mixers, a centrifuge, separation funnels, vacuum flasks, a millipore filter holder and filters, a lead pig for isotope storage, refrigerator for storing the proteins, a "walk in" type fume hood and a large array of laboratory glassware.

After subsequent difficulties in concentrating the labeled bacteriophages, albumin molecules from human serum were selected instead. These fall at the lower end of the size-range of viruses. They have a high molecular weight, spheroidal shape and a proteinaceous surface. They were commercially available, at high concentration, already labeled with Iodine-131; and, since so much research has been done with blood proteins, considerable literature was available on their chemical and physical properties.

The use of bacteriophages as originally planned could have been carried out employing normal virus assay techniques to trace their movement. Had this been done, one would have assayed the influents, effluents and washings from soil samples. There are, however, several advantages to using a radioactive tagging technique as contrasted to the assay method of tracing, the primary advantage being one of speed and precision. Counts of radioactivity on samples of influent or effluent solution can be quickly made without incubating the samples first. Also, a much more quantitative determination of the number of particles present is possible with the labeling technique since one is never sure how many particles were responsible for a particular plaque. One must also consider the possibility of die-off. If the virus particle dies, it is no longer possible to determine its location with the usual assay techniques. In analyzing the removal process, it is nevertheless desirable to know what becomes of such particles and a radioactive particle can be traced whether dead or alive until the point is reached where denaturation has progressed far enough that the radioactive nuclide dissociates from the protein particle. The amount of "die-off" in the makeup water can be determined, but this rate is not necessarily the same as the rate of "die-off" in the permeameter. This would be particularly true if the flow were occurring under partially saturated conditions since proteins would be in contact not only with soil surfaces and soil microorganisms but also air-water interfaces where adsorption (Adams, 1948) and possibly denaturation and subsequent die-off could occur.

When a gamma emitter such as Iodine-131 is used, not only is it possible to detect the presence of the particles in relatively thick slices of soil sectioned from the column, but it is also possible to make certain qualitative observations by externally scanning the permeameter with a scintillation detector. In some cases, the use of Phosphorous-32 or Sulfur-35 incorporated in vivo into the virus particle might be preferable to the use of Iodine-131 to avoid the possible denaturation caused by chemical labeling and the presence of Iodine. In either case, the use of a radioactive tracer seems highly preferable to the normal biological assay techniques.

3. Description of protein materials --

(a) Bacteriophage: The T-7 strain of Escherichia Coli phage was obtained from American Type Culture Collection, 2212 M. Street, N. W., Washington 7, D. C.

(b) Albumin: Radio-iodinated human serum albumin (I. H. S. A. MDV I. V) catalog number 200 was obtained from Nuclear Consultants Corporation, St. Louis 19, Mo.

C. Procedure for Studying Protein Infiltration.

1. Introduction of the particles -- Once the desired hydraulic conditions were established, the solution containing labeled albumin was either introduced through the inflow barrier in place of the water or was pipetted into the upper soil surface as a discrete charge. In the former case, a glass tee was connected to the inflow barrier. One end of the tee led to the Mariotte siphon. The other arm of the tee was connected to a glass sphere (about $2\frac{1}{2}$ cm. in diameter) open at each end. The other side of the glass sphere was connected to two leveling bottles (reservoirs similar to those used for capillary pressure-saturation measurements, see Appendix C). Thus the flow from the Mariotte siphon could be cut off and any protein present in the glass sphere could be introduced into the column at the prevailing capillary pressure by use of the leveling bottles. In cases where the labeled particles were pipetted as a discrete charge, the normal inflow of water was accomplished by a drip system or through a circumferential barrier, leaving the upper soil surface exposed. The drip system consisted of a flow metering device designed to maintain a steady flow of water into the column. The degree of saturation in the column adjusted automatically according to the flow rate. When the

capillary barrier and Mariotte siphon were used, the capillary pressure was controlled and the flow rate adjusted itself accordingly. Both systems performed satisfactorily.

2. Tracing the particles -- After the particles were introduced, the effluent was sampled at regular intervals to determine when particles first come through and to determine their concentration as a function of time. Effluent samples from the permeameter were collected in 13 x 100 mm. glass culture tubes. The concentration of the protein in the effluent solution was determined by measuring the radioactivity using a scaler and a small well detector made for test tubes.* During this time, the permeameter was continuously scanned from the outside to locate the position of the protein "wafer" and to determine the approximate distribution of particles at any particular time. The scanning apparatus is shown schematically in Figure 6. The probe was a Nuclear Chicago Model DS5-3 containing a one-inch diameter sodium iodide, thallium-activated crystal. This was inserted in a lead collimator and the entire unit was mounted on a moveable carriage which could be raised and lowered to enable the permeameter to be scanned. The signal from the detector was fed into a radiation analyzer,** the output of which went into a scaler***

---

*Baird Atomic Model 810 B, Scintillation Detector with Na 1, T 1 activated crystal. Baird Atomic Model 132, Scaler.

**Nuclear Chicago Model 1810. Single Channel Differential Pulse Height Analyzer.

***Nuclear Chicago Model 186, Decade Scaler.

and ratemeter* for readout. The ratemeter was connected in parallel with the scaler to serve as a check on the scaler reading and to provide an "instantaneous" estimate of activity.

Upon completion of a run, the column was removed from the apparatus and a series of 1/2 cm. slices was taken throughout its length. These were placed in 4 oz. paper cups to air dry. These samples were then weighed, and the amount of protein present was measured in a large scintillation well, equipped with another DS5 probe containing a 2 in. sodium iodide crystal. When this was used, it was connected to the radiation analyzer in place of the DS5-3 described above.

In most experiments the protein was metered into the permeameter through the inflow barrier and due to the nature of the apparatus, some protein was retained in the metering system making it difficult to determine how much actually entered the column. For this reason it was necessary to perform a graphical integration to determine how much protein was actually present in the column. The details of this calculation are given in Appendix D.

D. Procedural Modifications.

1. Change of soil structure -- Significant quantities of protein were retained by the media under conditions where no removal due to

---

*Nuclear Chicago Model 1620 B, Analytical Count Ratemeter sieving had been predicted. The following test was designed to show conclusively whether or not this removal was due to a sieving mechanism.

If removal was being accomplished by the sieving mechanism already described, the albumin molecules would be retained in the joints and crevices formed along the areas of contact between sand grains. By separating the individual grains and washing them over a filter capable of passing albumin but not soil, any protein so trapped would be released and would pass through the filter with the wash water. Approximately 700 mg. of IHSA* were added to a column of untreated Greeley sand during fully saturated steady-state flow followed by flushing of the column with distilled, filtered water until the effluent count was the same as background. Thus no further leaching would have been expected. The column was then dissected and the usual 1/2 cm. slices were taken. This time, however, the soil slices were placed in a filter holder and stirred continuously to keep the sand grains separated while washing with three successive 50 ml. qualtities of distilled water over an 0.45 micron millipore filter. The filters were first checked to be sure that the albumin molecules would pass through. The soil was washed from the filter into paper cups and the activity on the soil, in the filter and in the filtrate was measured in the well detectors previously described. Comparison of filtrate readings with media readings revealed how much albumin was sieved or trapped and how much was retained by some other mechanism.

---

*Radioiodinated human serum albumin

2. Pre-treatment of soil surface -- Some of the protein retention that occurred under conditions where none was expected was thought to be a result of adsorption. It was postulated that such adsorption was minor and that pre-treating the soils with protein solutions prior to introduction of the labeled albumin would satisfy their adsorptive capacity. Investigation of the sieving process could then proceed as previously described.

Pre-treatment was accomplished by vacuum saturating the columns in solutions of albumin or blood protein instead of plain water. This was usually augmented by allowing an additional 500 mls. of protein solution to flow through the column prior to the introduction of the labeled material.

3. "Exchange phenomena" -- To gain further insight into the nature of the retention mechanism, an experiment was devised to show how much of the albumin retained might be released as more albumin was added. A column of White sand was pre-treated by vacuum saturation with IHSA followed by the flow of an additional 450 mls. of 0.10% IHSA solution in an attempt to saturate the adsorptive capacity of the soil with labeled albumin. Following this, several liters of distilled water were flushed through the column until the effluent count was the same as background. Then a quantity of unlabeled albumin was added to the column to see how much of the labeled albumin would be displaced.

E. Supplementary Experiments.

1. Correlation of retentive capacity with specific surface area -- A series of static tests were conducted in beakers using various coarse-grained materials to compare their retentive capacities. The materials used were:

Greeley sand (heat treated) - same as previously described.

Greeley sand (acid-treated) - same as above, but mixed with sodium hexametaphosphate and washed over a 300 mesh sieve to remove the clay and organic matter. It was then soaked in 6 N HCl to remove any iron present and thoroughly rinsed to removal all the acid.

Greeley sand - same as previously described, but not treated in any way.

White sand - same as previously described.

Crushed quartz - This was made by crushing quartz pebbles in a burr mill. Then the material was treated with 6 N HCl to remove the iron. Following this, it was sieved, separated and recombined to provide the same particle size distribution as the Greeley sand.

Dune sand - a uniform wind-blown sand with somewhat rounded particles, containing quartz and feldspar and having a particle density of 2.67 gms./cm^3. This material was obtained from Great Sand Dunes National Monument.

Silicon carbide - This was 80 mesh uniform material obtained from a laboratory supply house.

Alundum - 80 mesh uniform material obtained from a laboratory supply house.

Equal weights of the materials were placed in clean beakers and equal volumes of 0.10% radio-iodinated human serum albumin were added to the beakers in the ratio of 1 gm. of solution per gm. of soil. The samples were stirred gently for five minutes and then washed over 0.45 micron millipore filters with stirring to remove any trapped protein. Then the soil was placed in paper cups, air-dried, weighed and the activity measured in a well detector. These values of retention were then compared with the specific surface area of the materials obtained by an ethylene glycol method (Bower and Goertzen, 1959), courtesy of W. A. Norvell and C. A. Bower, Agricultural Research Service, U. S. Salinity Lab., Riverside, Cal.

2. Hydraulic properties of materials -- A series of separate experiments were conducted to determine the hydraulic properties of the porous materials used in the protein infiltration experiments. These tests included the measurement of bubbling pressure, residual saturation, pore-size distribution index, air-entry pressure and saturated permeability. All such data were taken only for the drainage cycle and procedures and results are presented in Appendices B and C.

F. Precautions.

Recognizing that proteins such as albumin are rather easily denatured and are also subject to bacterial action, the following precautions were taken:

Stock solutions of IHSA and HSA were kept refrigerated and were handled only with sterile pipettes.

The soils used in the permeameters were sterilized by heat treatment at 1400° F.

The acrylic permeameter sections were washed and rinsed in alcohol prior to use.

All water used in the experiments was distilled and doubly filtered through 0.22 micron filters.

Albumin solutions were diluted gradually to lessen the possibility of denaturation.

CHAPTER V

RESULTS AND CONCLUSIONS

A. Discussion of Experimental Results.

The data (resulting from dissection of the columns and subsequent measurement of the albumin retained in the $\frac{1}{2}$ cm. slices) were processed as follows: The mean reading in counts per 30 seconds was obtained for each slice and from this the background count was subtracted. All readings were then divided by the maximum reading and these were then plotted as $\frac{\theta}{\theta_{max.}}$ versus depth. By following the procedure outlined in Appendix D, an estimate was made of the amount of protein retained by the column in per cent of the amount introduced. The data were used to determine the functional relationship between $\frac{\theta}{\theta_{max.}}$ and depth and this relationship was analyzed in an attempt to determine what mechanism of retention was acting.

It seemed reasonable to assume that $\theta_o \approx \theta_{max.}$ since the theory predicts that the maximum value of θ should occur at the soil surface; i. e., $y = 0$. In most cases, the maximum value of θ did not occur at the soil surface and this was attributed to disturbance of the surface resulting from insertion of the inflow barrier into the column prior to making the runs and the disturbance resulting from pushing the soil out of the acrylic sections when cutting the $\frac{1}{2}$ cm. slices.

The values of θ obtained by scanning the column externally were of particular use in determining the location and number of slices to be made when the column was dissected. Because of the poor resolution obtained by scanning, they were not of quantitative value. Figure 8 shows a comparison of the activity obtained by scanning and sectioning. The readings were influenced very much by the total activity near the detector although the detector was well shielded and a collimator was used. This is evident by noting that the scanning procedure gave much lower values near the surface of the column where the effective aperture of the collimator actually took in part of the region above the soil surface where no activity was present. The data obtained by sectioning the columns are tabulated in Appendix E in terms of mean count minus background count for each slice. Pertinent hydraulic and physical conditions are also listed for each run. The values of θ are based on total activity per 6 gm. dry sample of soil.

1. Evidence contradicting the sieving theory -- Equations (21) and (22) predict the value of the exponent for the relationship between θ and y. When all the experimental runs were taken into consideration, it was found that the maximum value of the exponent occurred for G. E. No. 8 sand. The runs made with this material were at a degree of saturation of 1.0. Using equation (22), the term

$$\frac{1}{w\psi}\left(\frac{P_b}{P_c'}\right)^{2+\lambda}$$

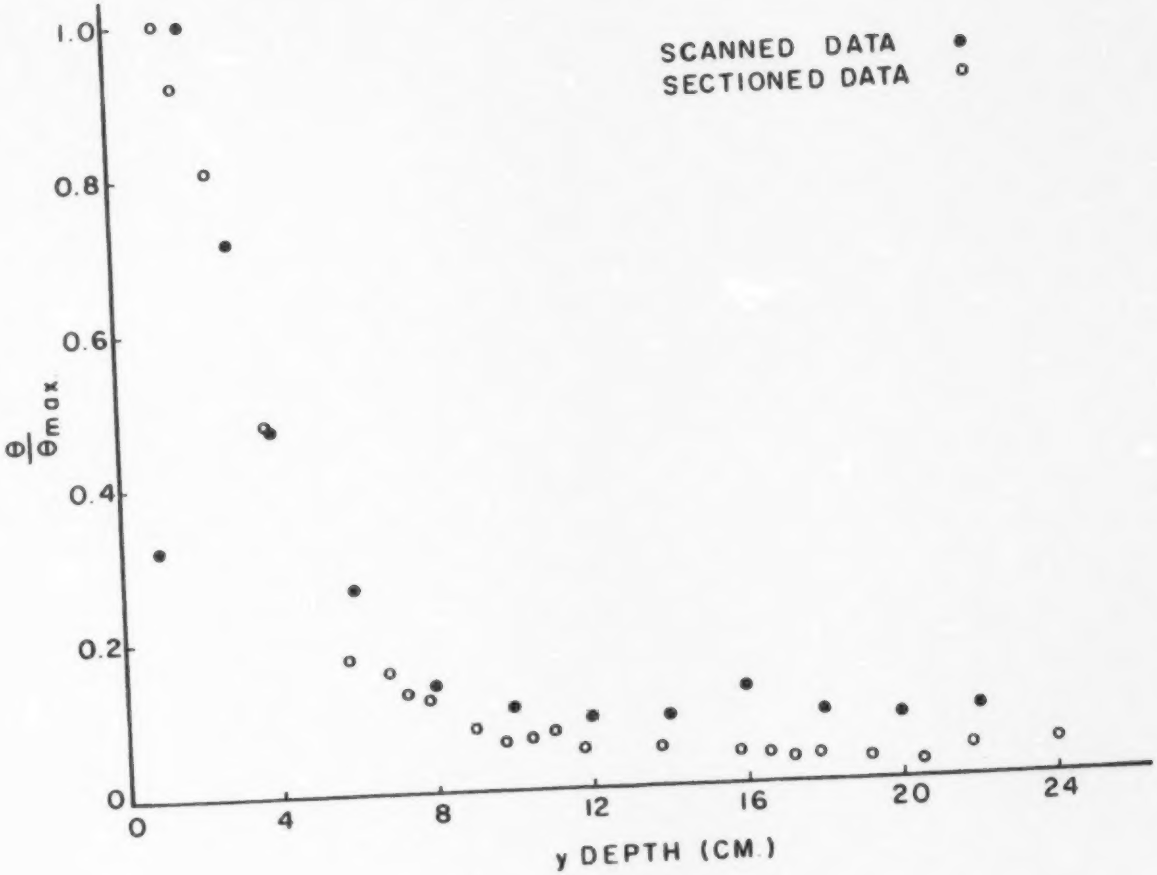


Figure 8. Comparison of data for the distribution of albumin obtained by scanning with that obtained by sectioning

can be written as

$$\frac{1}{w\,\psi}\left(\frac{r_e}{\frac{2\sigma}{P_b}}\right)^{2+\lambda}$$

since $P_c' = \frac{2\sigma}{r_e}$ (see theory). Approximating r_e by $\frac{d_p}{2}$, the effective radius of an albumin particle, gives

$$\frac{1}{w\,\psi}\left(\frac{d_p}{\frac{4\sigma}{P_b}}\right)^{2+\lambda}$$

The size of albumin molecules is about 15 millimicrons; $\frac{P_b}{\gamma}$ for G. E. No. 8 sand is about 85 cm. of water and λ is about 1.5. Assuming a wafer thickness w of about .01 cm. and value of $\psi = 1.0$, the value of the exponent is approximately 10^{-10}. If sieving were the mechanism responsible for the removal of protein particles, the slope of a semi-logarithmic plot of $\frac{\theta}{\theta_{max.}}$ vs. y would be negligible. Data for runs 4 and 5 appear on such a plot in Figure 9. The slopes are not negligible but are of the order of magnitude 10^{-1}. The exponents were calculated also for the other materials. The value for Greeley sand was 10^{-13} and for White sand 10^{-21}. Plots of log. $\frac{\theta}{\theta_{max.}}$ vs. y showed that if one could approximate such data with a straight line, the slopes were also of order of magnitude 10^{-1}.

Further evidence contradicting the sieving hypothesis was obtained on a purely experimental basis. Some of the removal could have been due to sieving, although perhaps only a very small part. An experiment was conducted to determine exactly how much removal was due to sieving and how much was a result of some other mechanism. The

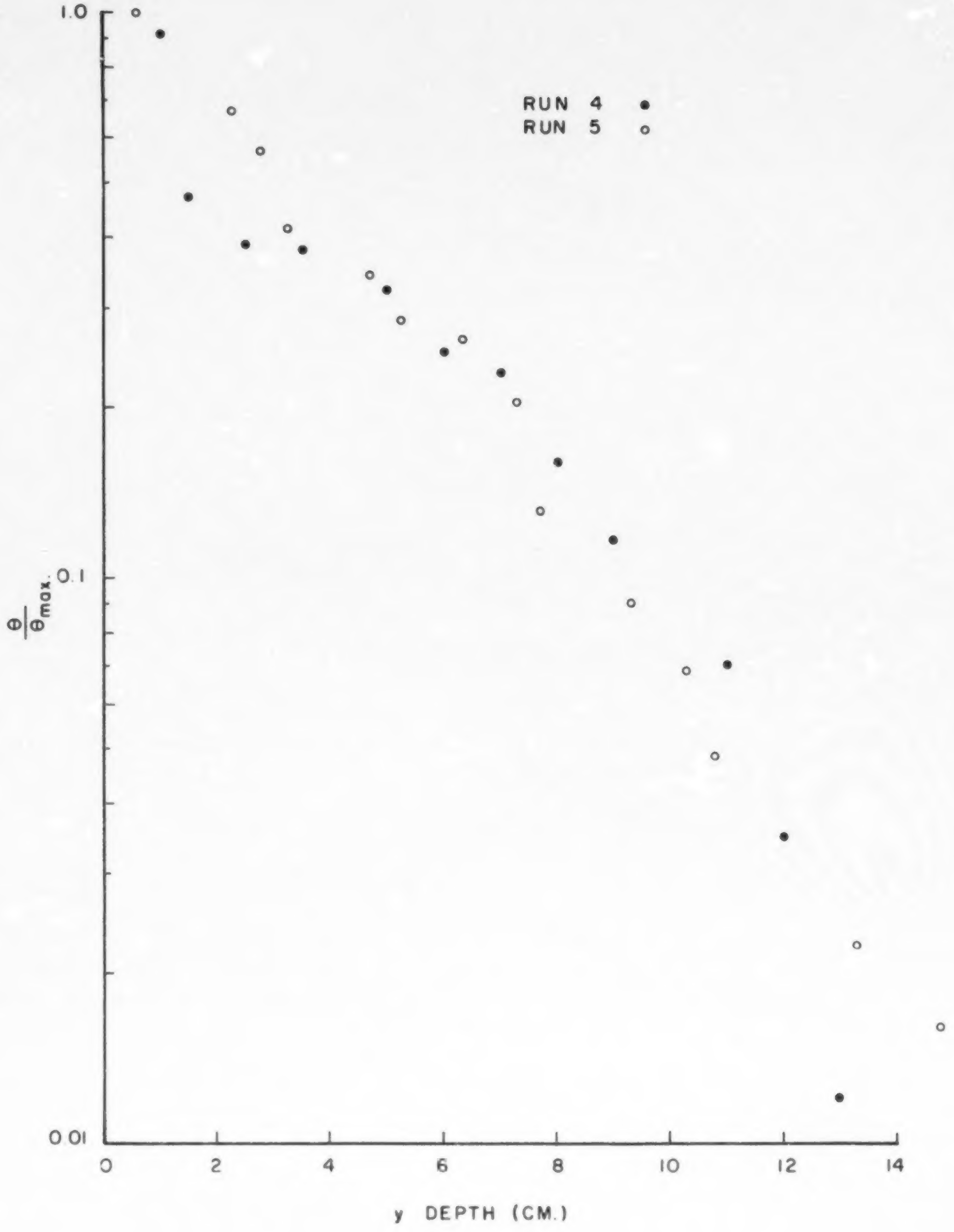


Figure 9. Distribution of albumin retained in a soil column

usual procedure was modified so that when the column was sectioned the sand grains were separated and washed over a filter (run 15). Any albumin caught between grains would be released and would pass through the filter. Table 2 shows the amount of albumin retained on the soil together with the amount washed off. Of the original 700 mgs. of albumin added to the column, 196 mgs. were retained and subsequent washings removed only 0.3 mgs. It was concluded that retention of protein for these materials was not due to sieving but to some other mechanism, adsorption being the most likely.

2. Evidence supporting the adsorption theory -- If equation (2) is integrated from $y = y_1$ to $y = y_2$, an equation is obtained for concentration as a function of depth:

$$\frac{C_2}{m} = \frac{C_1}{m} \int_{y_1}^{y_2} \theta \, dy \quad .$$

The integral on the right hand side can be evaluated graphically, and by assuming a value of $\frac{C_1}{m}$ at a particular location in the column, values of $\frac{C_2}{m}$ can be obtained corresponding to certain values of θ. Because of the difficulties already mentioned in evaluating θ at the soil surface, it was not possible to begin this calculation at $y = 0$. By starting at the depth corresponding to $\theta = \theta_{max.}$, an approximation was obtained for the concentration at any other point in terms of the concentration at that value of y. The resulting plot of $\frac{C_2}{m}$ vs. θ is defined for purposes of this study as a pseudo-isotherm. Equations (23) through (26)

TABLE 2

Distribution of retained material subsequent to separation of sand grains and repeated washing.

(Run 15)

Depth (cm.)	Albumin Retained mg. /gm. of soil	Albumin Washed Off mg. /gm. of soil
0 - 0.5	.650	.007
0.5 - 1.0	.577	.043
1.0 - 1.5	.532	negligible
1.5 - 2.0	.515	↓
2.0 - 2.5	.490	
2.5 - 4.0	.479	
4.0 - 4.5	.500	
5.5 - 6.0	.490	
7.0 - 7.5	.500	
8.0 - 8.5	.517	
9.0 - 9.5	.514	
10.0 - 10.5	.486	
11.0 - 11.5	.486	
12.0 - 12.5	.465	
13.0 - 13.5	.449	
14.0 - 14.5	.458	
15.0 - 15.5	.442	
16.0 - 16.5	.445	
17.0 - 17.5	.434	
18.0 - 18.5	.441	
19.0 - 19.5	.386	
20.0 - 20.5	.384	
21.0 - 21.5	.361	
22.0 - 22.5	.347	
23.0 - 23.5	.352	
24.0 - 24.5	.371	
26.0 - 26.5	.367	
27.0 - 27.5	.353	

are examples of theoretical adsorption isotherms. Since experimental adsorption isotherms had not been run on these materials, there was no way of computing the adsorption constants such as k, n, a, and b appearing in these equations or the distribution equations (27) through (30). If the shape of the pseudo-isotherms were assumed to be the same as the real isotherms, the functional relationship between $\frac{\theta}{\theta_{max.}}$ and y could be predicted qualitatively through equations (27) - (30). Subsequent empirical fitting of the data showed that the function which gave the best fit was the one predicted by the pseudo-isotherm. The experimental conditions for runs 3, 4, 5, 6, 12 and 15 closely approximated those conditions set forth in the theoretical adsorption model. Using the method of least squares, the functional relationship predicted by the pseudo-isotherm was fitted to these data. The results are plotted in Figures 10 through 15. The values of $\frac{\theta}{\theta_{max.}}$ are plotted for the midpoints of the $\frac{1}{2}$ cm. slices. The qualitative shape of the pseudo-isotherm is stated in each figure together with the general functional relationship between θ and y for that particular run as predicted by the pseudo-isotherm. This indicates that the distribution of albumin can be predicted at least qualitatively by the adsorption model.

It was originally expected that adsorption would be negligible for sands and that by pre-treating the sands with various protein solutions, their adsorptive capacity could be satisfied. The columns could then be desaturated until a condition was reached such that P_c was large enough that removal by sieving would start to occur. But, even under

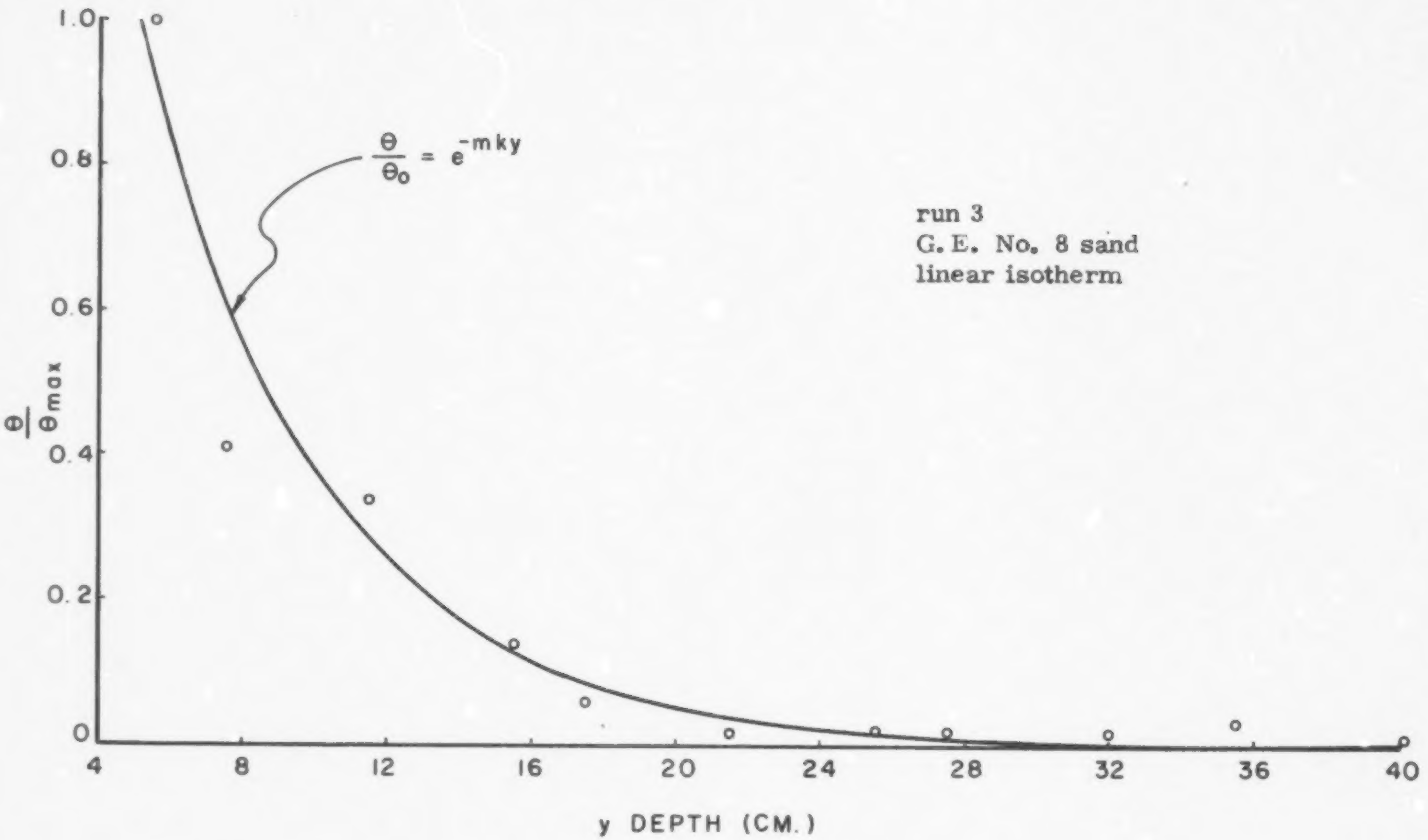
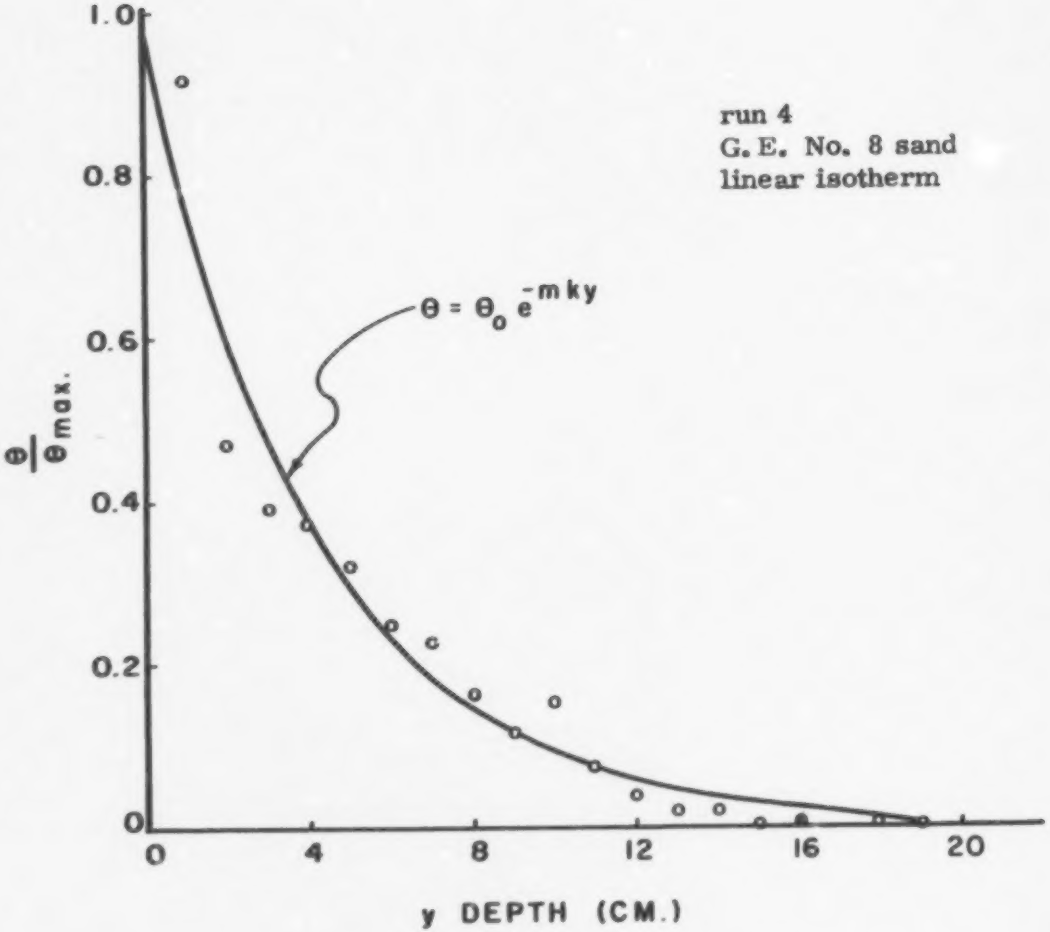
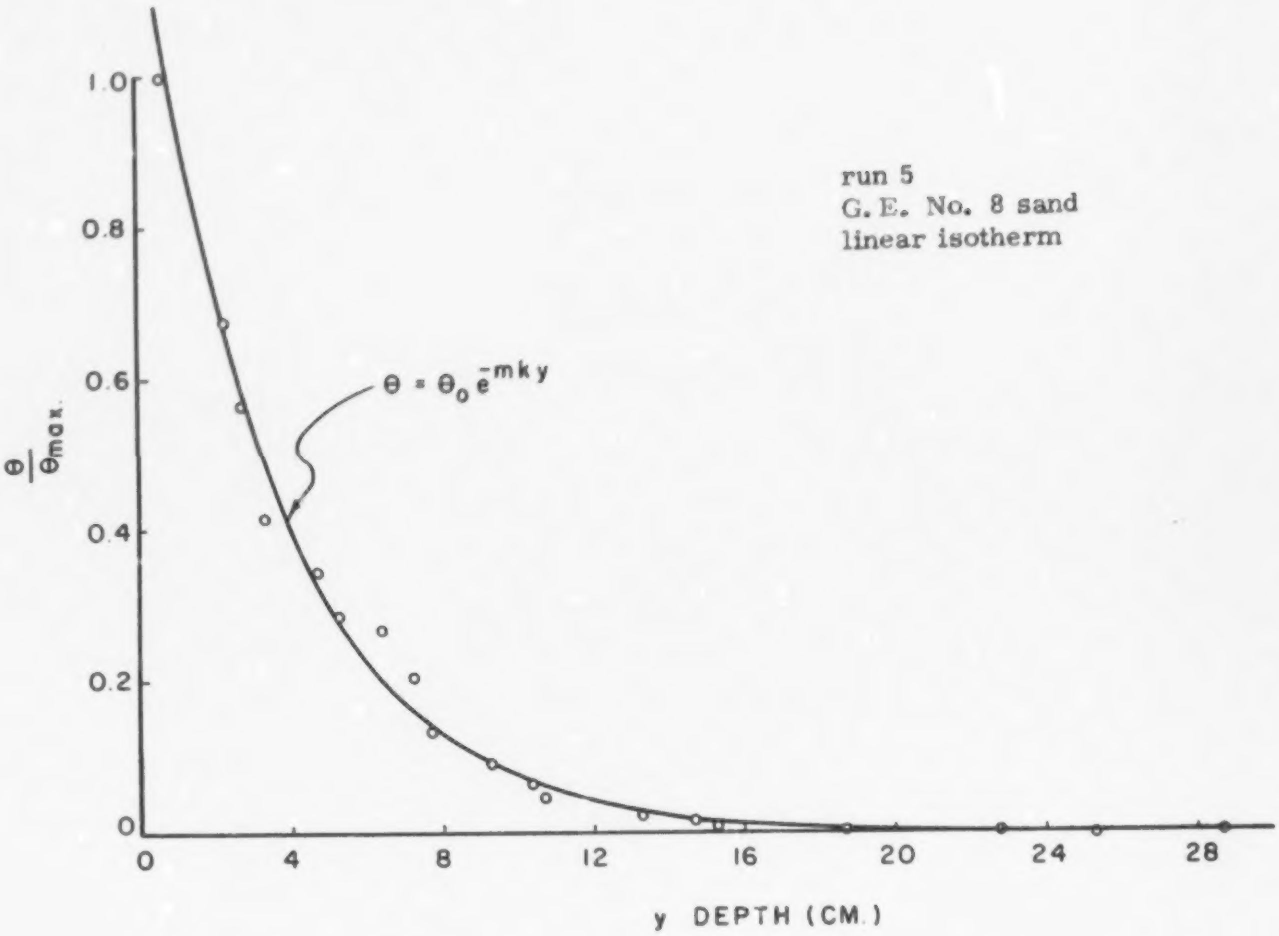


Figure 10. Distribution of albumin retained in a soil column



run 4
G. E. No. 8 sand
linear isotherm

Figure 11. Distribution of albumin retained in a soil column



run 5
G. E. No. 8 sand
linear isotherm

Figure 12. Distribution of albumin retained in a soil column

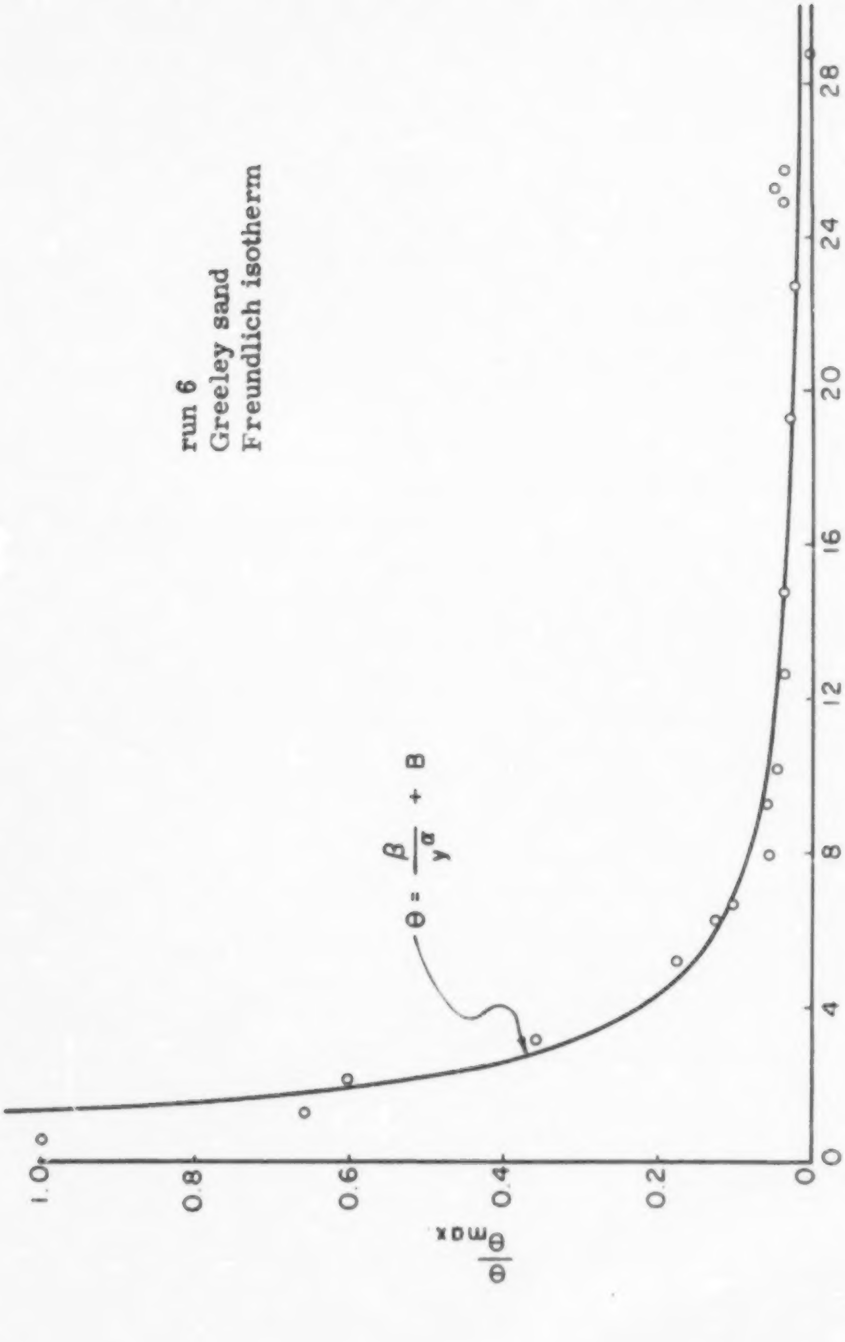


Figure 13. Distribution of albumin retained in a soil column

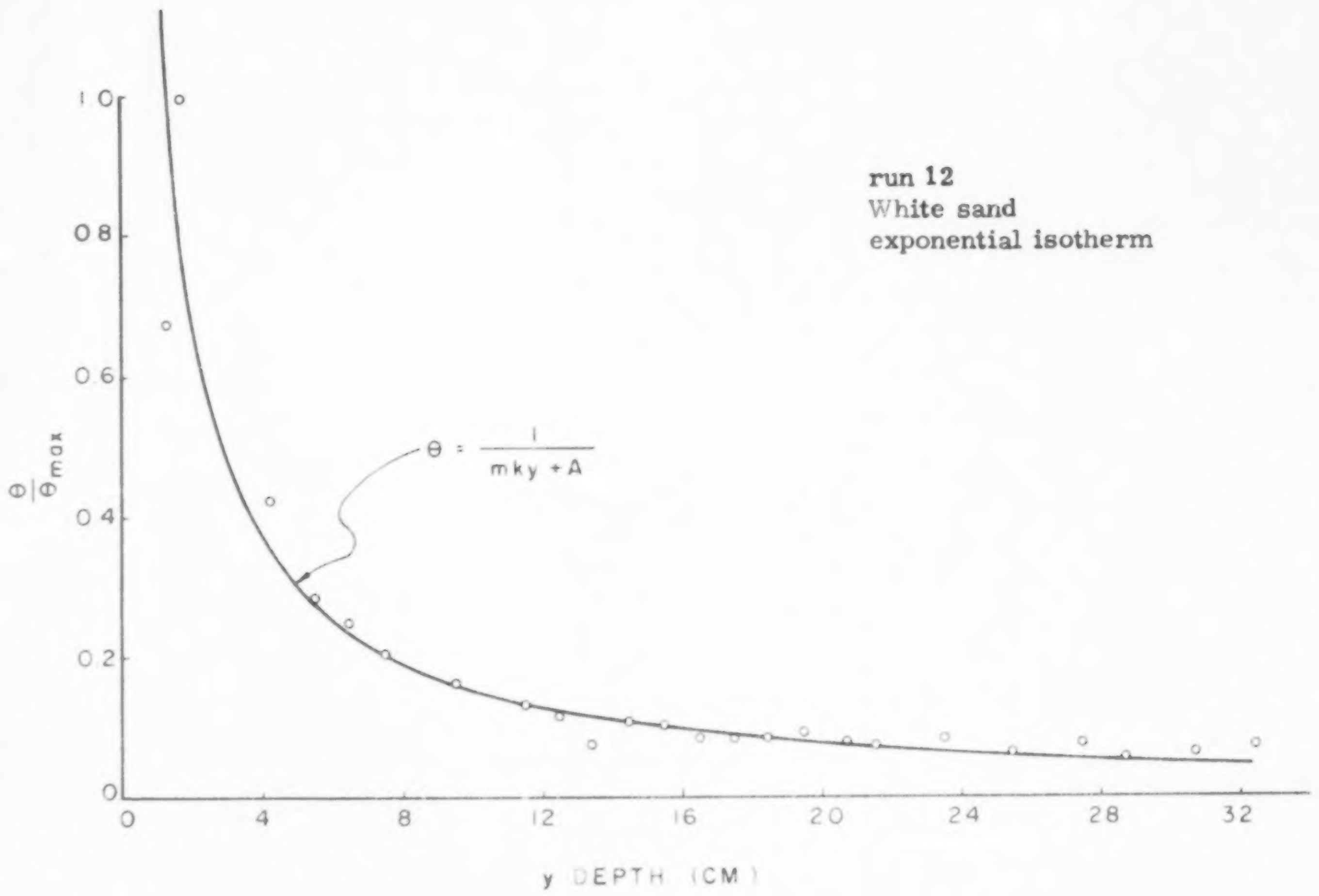


Figure 14. Distribution of albumin retained in a soil column

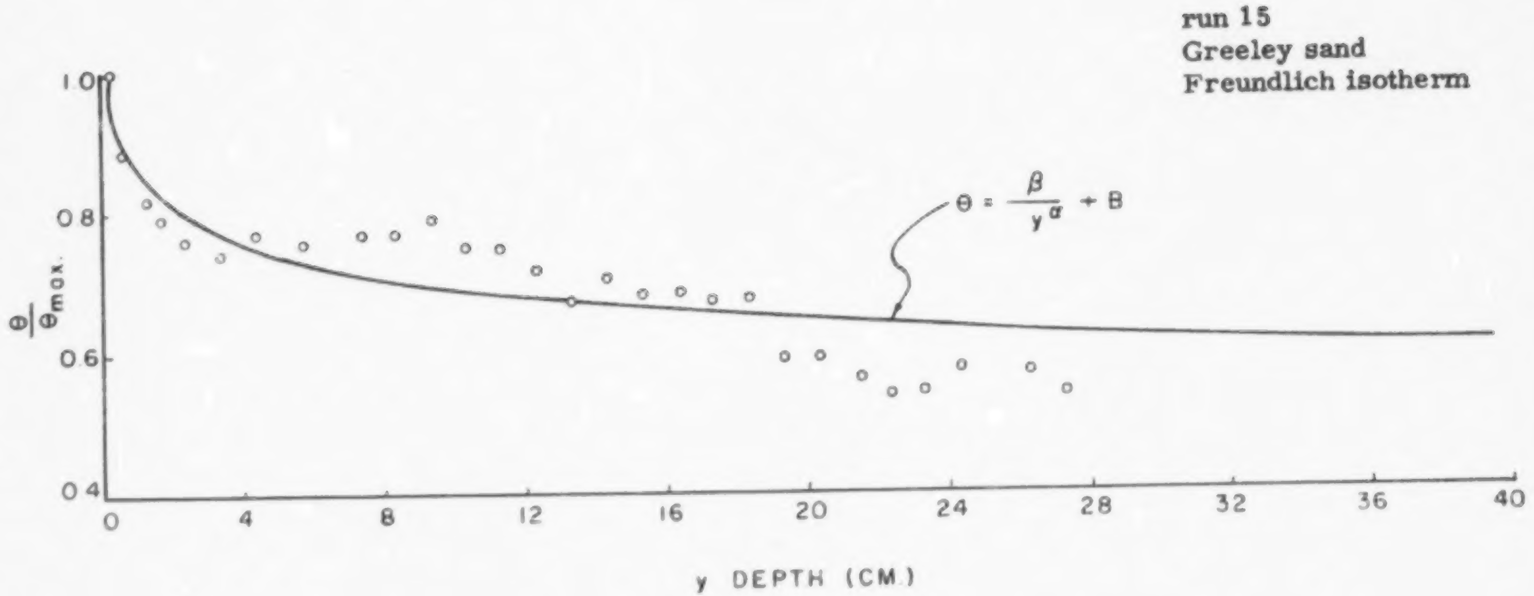


Figure 15. Distribution of albumin retained in a soil column

saturated conditions, the pre-treated columns were able to retain considerable quantities of the labeled albumin subsequently introduced.

Table 3 shows the effect of pre-treatment on protein retention. The most obvious fact to be concluded is that pre-treatment did not saturate the retentive capacity of the soil as had been anticipated and that calf-serum is less effective than human serum albumin in reducing the retentive capacity. Since the serum contains most of the other blood proteins including the oppositely charged particles, this result seems reasonable. A second less obvious conclusion is that sandy soils, even those containing no clay, are able to retain a surprising amount of protein, a result which was totally unexpected.

The theoretical equations predicting the distribution of albumin as a function of depth were derived assuming that the soil had not been previously exposed to albumin. However, since the removal of albumin under the conditions of pre-treatment was suspected as also being due to adsorption, it was thought that the equations might predict the distribution of the labeled albumin in a qualitative way. Adsorption in this case might have been occurring on the soil or on the unlabeled protein used in pre-treatment or on both. The resulting data appear in Figures 16 through 21. The only explanation for Figure 18 is that during the course of the run, the outflow barrier became plugged which might account for the accumulation of albumin in the lower portion of the column. No attempt was made to fit a functional relationship to the data for this run. From the appearance of these distributions,

TABLE 3

Effect of Pre-Treatment on Retention of Labeled Protein

Run No.	Soil Material	Flow Rate (cm. /sec.)	Pre-Treatment	Additional Protein Added	Amt. of Radioactive Albumin Added (mgs.)	Percent Retention
6	Greeley sand	1.10×10^{-2}	None	None	5. 5	87
7	Greeley sand	1.25×10^{-2}	Calf Serum	None	5. 5	51
8	Greeley sand	1.15×10^{-2}	H. S. A. *	500 mgs. of H. S. A.	5. 5	46
12	White sand	1.95×10^{-2}	None	None	2. 4	58
11	White sand	1.59×10^{-2}	Calf Serum	500 mgs. of H. S. A.	2. 4	35
9	White sand	1.78×10^{-2}	H. S. A.	500 mgs. of H. S. A.	2. 4	18

*H. S. A. - Human Serum Albumin 0. 05% Solution.

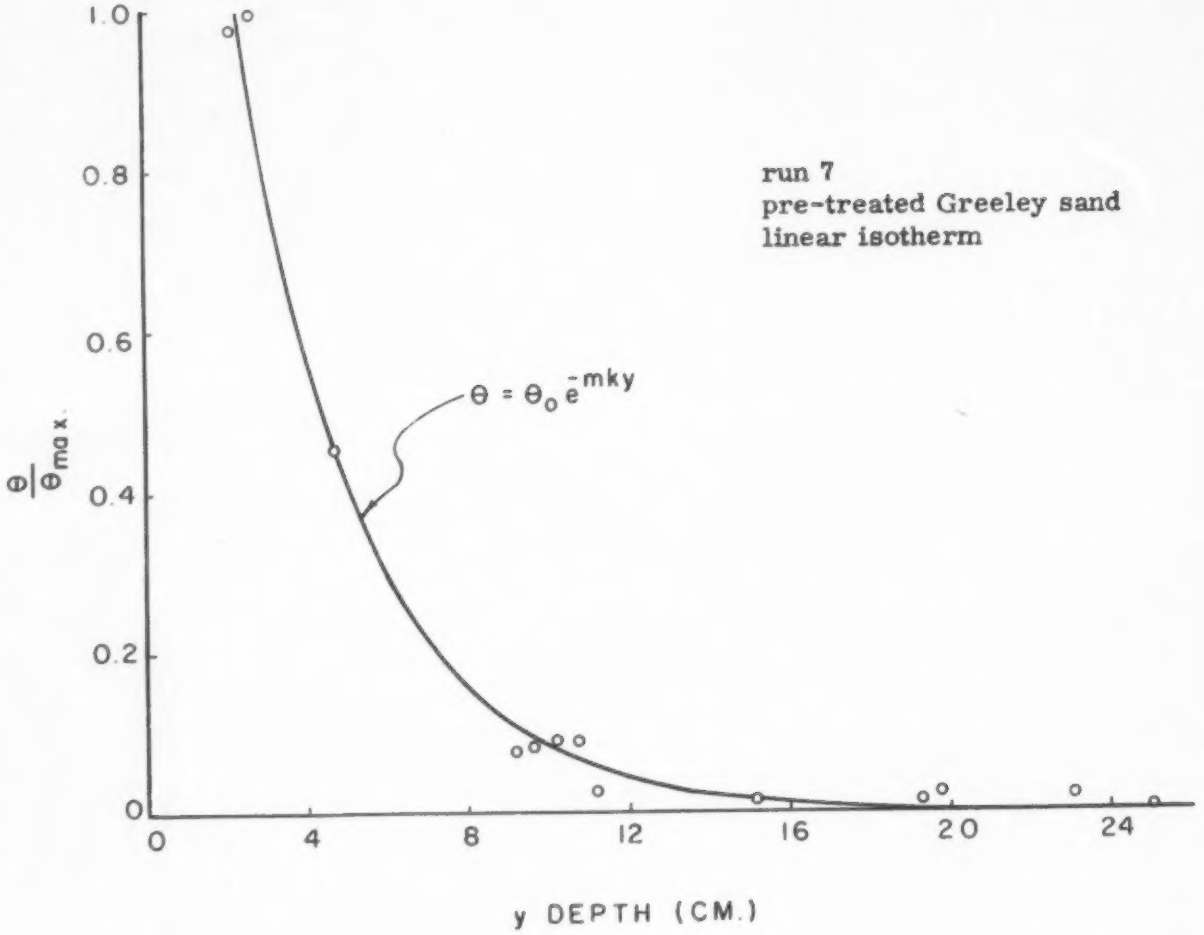
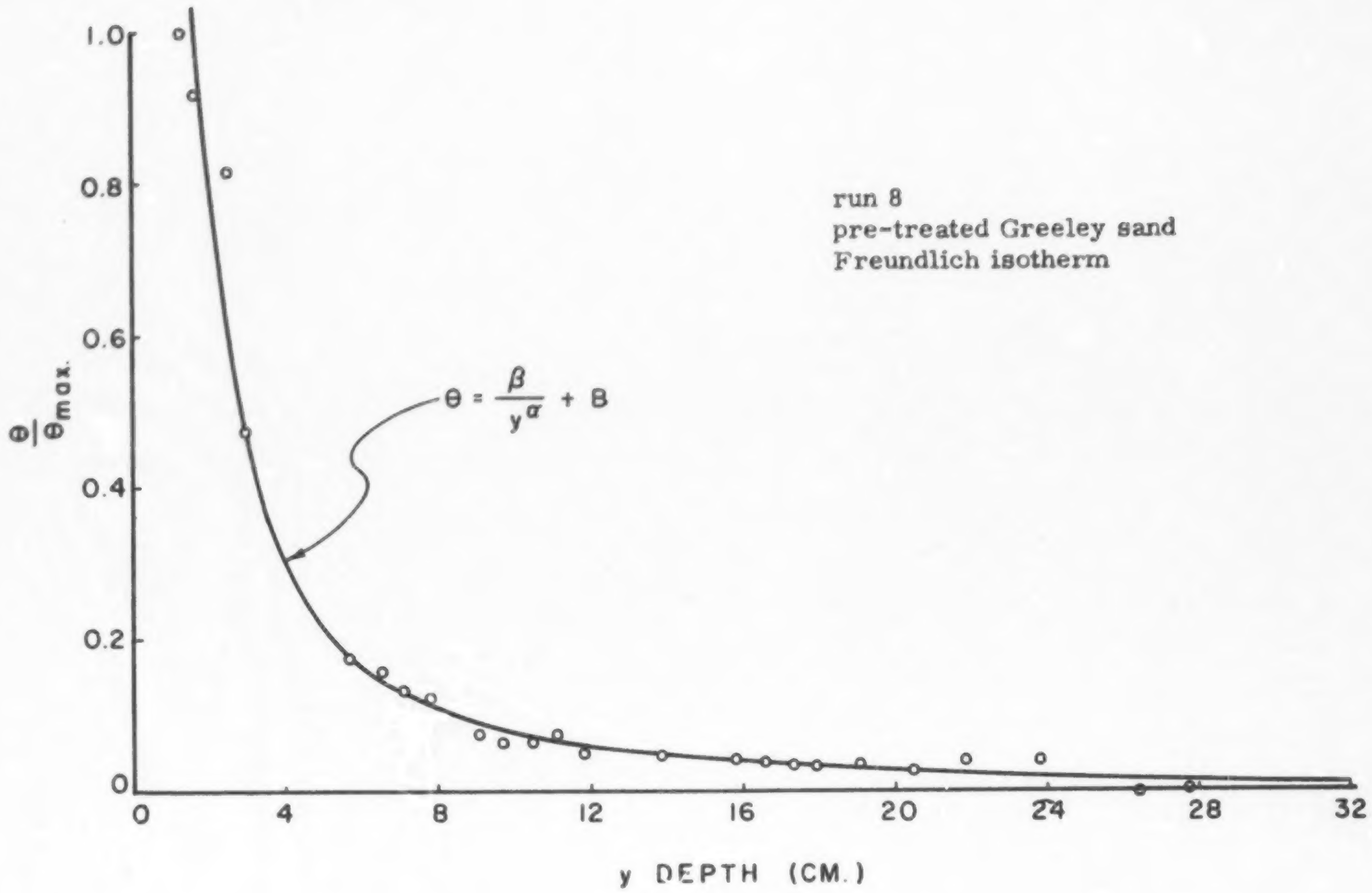


Figure 16. Distribution of albumin retained in a soil column



run 8
pre-treated Greeley sand
Freundlich isotherm

Figure 17. Distribution of albumin retained in a soil column

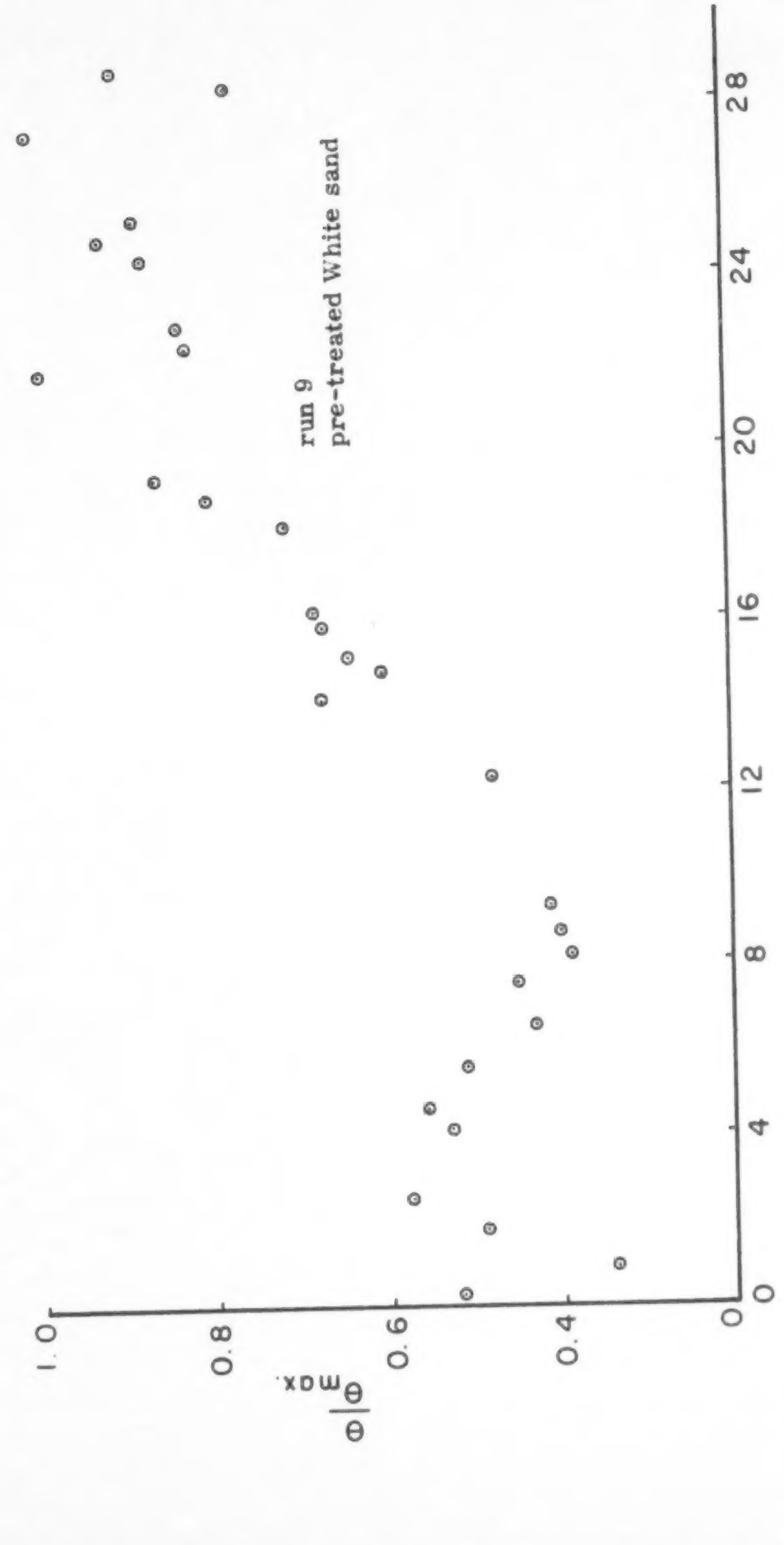


Figure 18. Distribution of albumin retained in a soil column

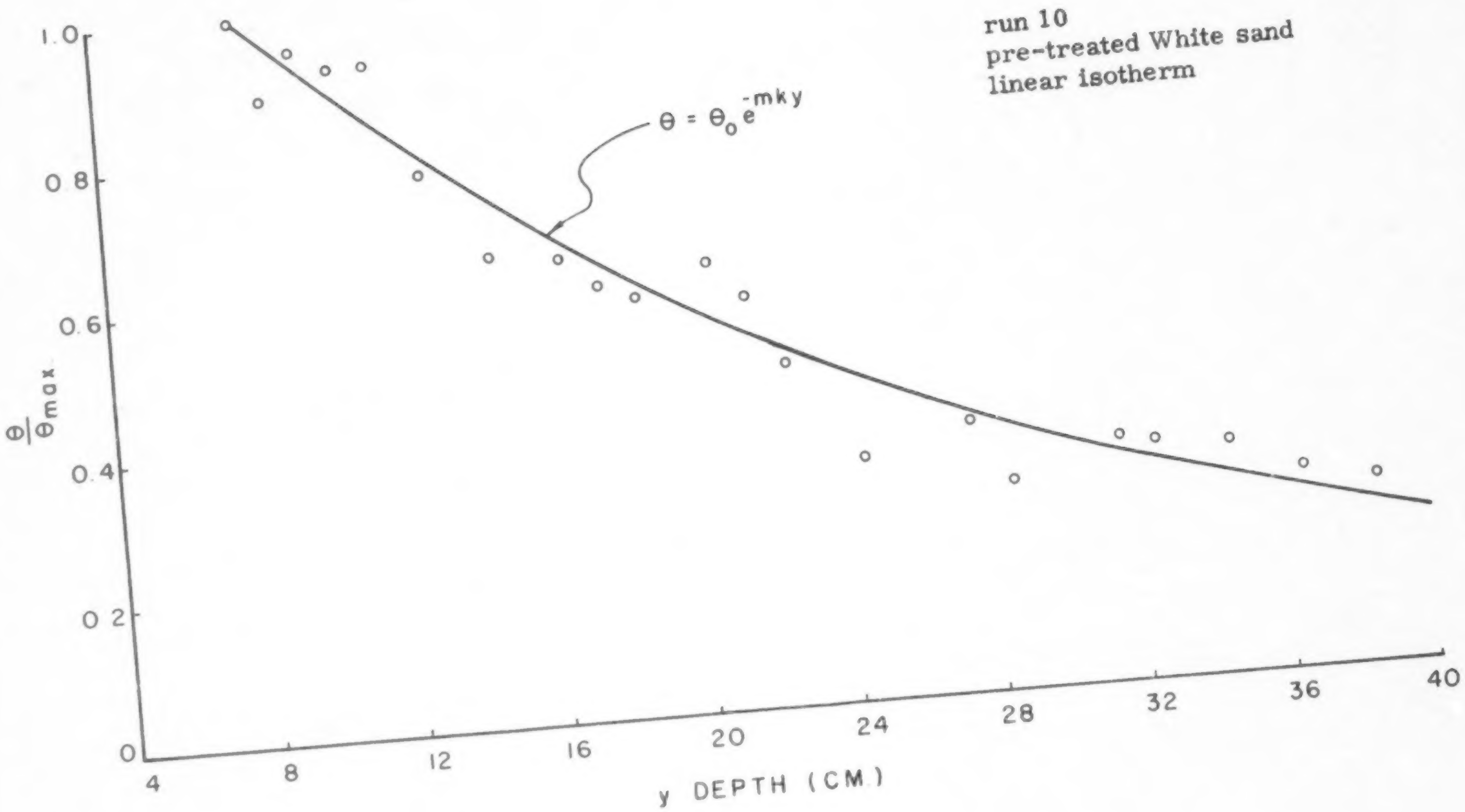


Figure 19. Distribution of albumin retained in a soil column

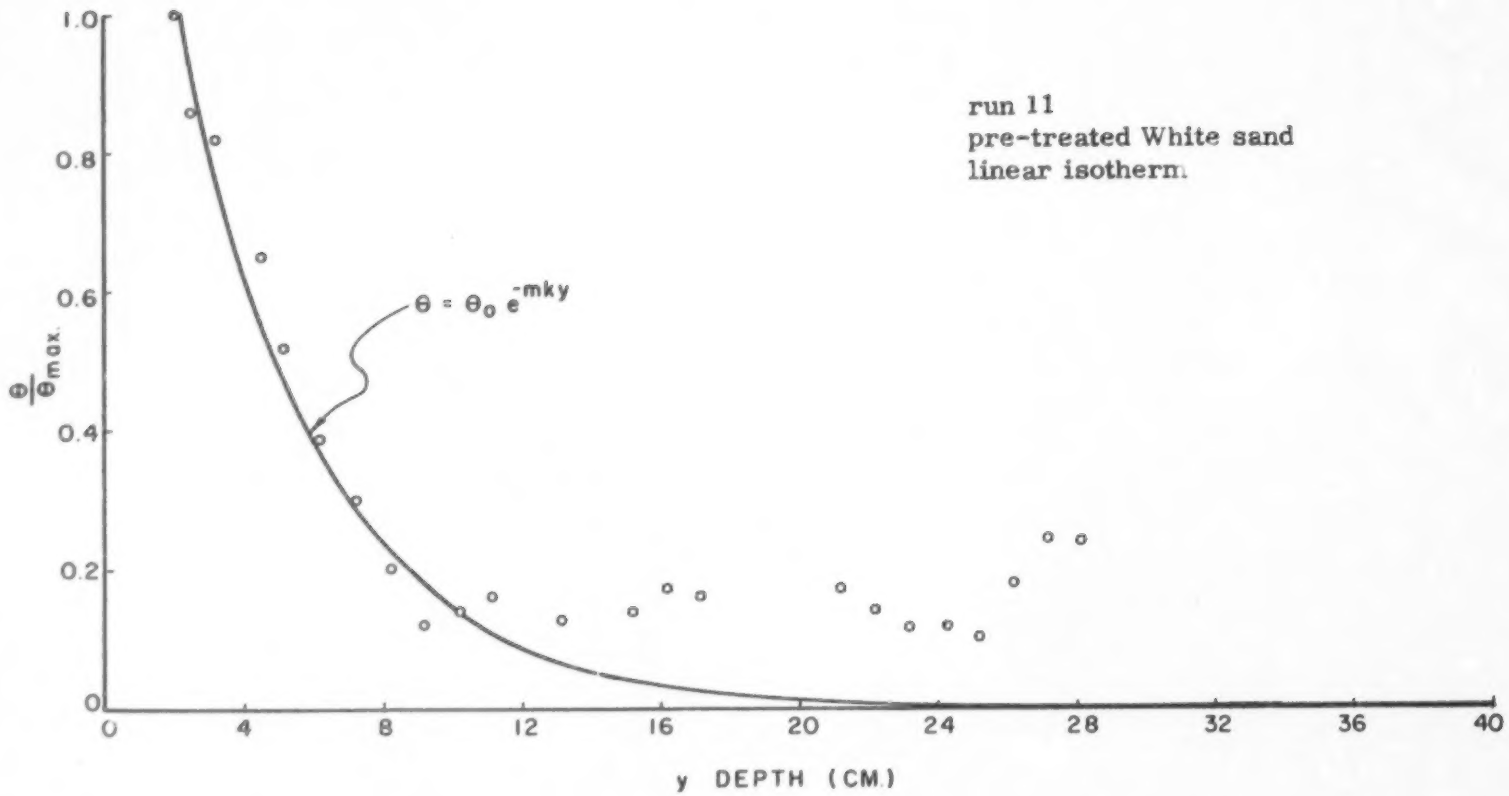


Figure 20. Distribution of albumin retained in a soil column

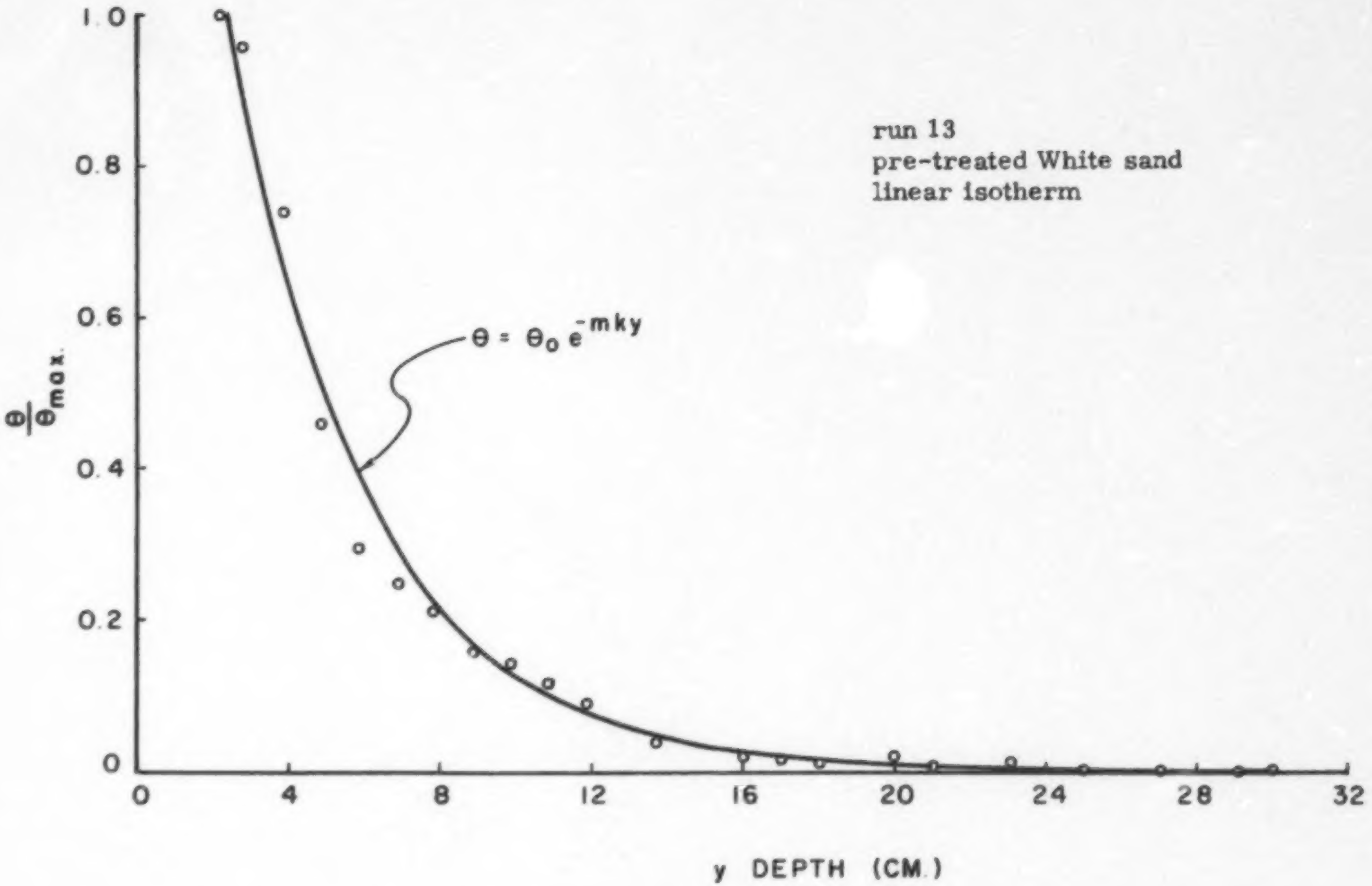


Figure 21. Distribution of albumin retained in a soil column

it would seem that even for the pre-treated columns, the retention was caused by some kind of an adsorption process.

If removal were due to adsorption, the surface properties of the porous materials (in particular the specific surface area) would be an important factor. A series of tests were conducted in which a variety of soil materials were placed in beakers containing solutions of radioactive albumin. The beakers were stirred and the soil materials were washed several times over a fine filter. The amount of radioactive albumin retained by the soil particles was then measured in a well detector and the results were compared with the specific surface area as measured by an ethylene glycol retention method. The results appear in Table 4. Greeley sand was the only material used which contained clay and the large increase in retention for the Greeley sand as compared to the other materials is apparent. The heat-treated Greeley sand also contained clay but in a structurally altered form. Its effect on protein retention is still quite marked. Retentive capacity seems to be closely related to the surface area which further supports the adsorption theory. Clay content seems to be the most important factor. This is further substantiated by considering the tabulated data for runs 4, 5, 6 and 12 in which no pre-treatment was involved. These are presented in Table 5.

It is known that G. E. No. 8 sand contained the most clay. The Greeley sand was second and the White sand contained no clay. Clay content affects both the surface properties and the hydraulic properties

TABLE 4

Retentive Capacity and Specific Surface

	Retained Protein mg. albumin/gm. of soil	Surface Area* M^2/gm.
Silicon carbide	0	0.2
Crushed quartz	0.06	0.1 - 0.2
Alundum	0.10	0.1
Greeley sand (acid treated)	0.103	3.2
White sand	0.117	0.4
Dune sand	0.16	12.7 - 14.5
Greeley sand (heated to 1400°F)	0.458	1.9
Greeley sand	0.571	19.0

*Specific Surface Area data were furnished through the courtesy of W. A. Norvell and C. A. Bower, Agricultural Research Service, U. S. Salinity Laboratory, Riverside, California.

TABLE 5

Effect of Soil Type on Total Retention $S_e = 1.0$

Run No.	Soil Material	P_b/γ (cm.)	η	Flow Rate / A (cm. /sec.)	Radioactive Albumin Added (mg.)	Protein Retention (Percent)
4	G. E. #8 sand	85	6. 5	1.18×10^{-3}	1. 38	92
5	G. E. #8 sand	85	6. 5	0.80×10^{-3}	5. 85	96
6	Greeley sand (heat-treated)	36	8. 3	1.10×10^{-2}	5. 50	87
12	White sand	40	15. 0	1.95×10^{-2}	2. 40	58

of a porous medium. Because of the complex inter-relationships among these factors it was not possible to separate out the various effects. The soils, however, are listed in order of decreasing clay content and their ability to retain protein seems to be directly related to their clay content.

In an attempt to learn more about the nature of the suspected adsorption, run 14 was conducted to determine if any sort of exchange was taking place. The column was first pre-treated by filling it with radioactive albumin. It was then rinsed with clear water until no albumin came through in the effluent. When 650 mgs. of unlabeled albumin were subsequently added, the column released 11. 5 mgs. of the labeled albumin. Dissection of the column revealed that 244 mgs. of radioactive albumin had been initially retained by the column before the unlabeled albumin was introduced. This indicates that a process somewhat analogous to ion-exchange might have occurred. The distribution of the retained albumin was plotted in Figure 22. No attempt was made to fit the distribution with a mathematical formula since the labeled albumin was not introduced as a slug or wafer and the conditions of the experiment did not meet the assumptions made in the theoretical development.

The mathematical models predicting protein removal by adsorption show that θ varies inversely with the degree of liquid saturation of the soil. On would expect, therefore, a greater amount of protein to be retained under partially saturated conditions. This is clearly confirmed by the data in Table 6.

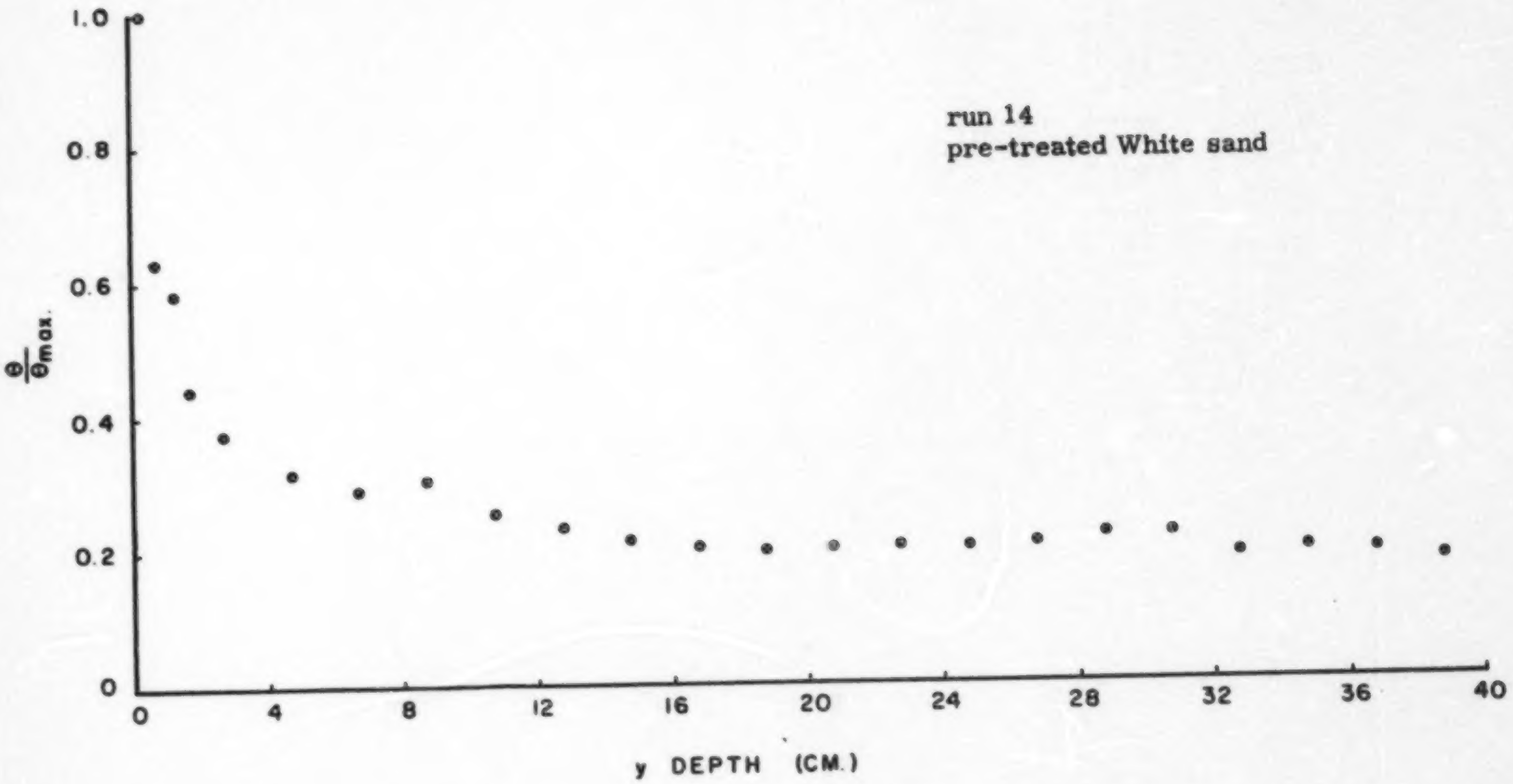


Figure 22. Distribution of albumin retained in a soil column

TABLE 6

Effect of Degree of Saturation on Total Retention

Run No.	Degree of Saturation	Percent Retention
11	1.00	35
10	0.89	75
13	0.81	93

B. Conclusions.

(1) Under the conditions maintained in these experiments, removal of albumin molecules from suspension by a sieving process was negligible.

(2) The primary mechanism by which albumin was removed from solution under the conditions of this investigation was some type of adsorption, the exact nature of which is not understood.

(3) The theoretical analysis based on an adsorption model predicted the shape of the albumin distribution curves qualitatively.

(4) Materials highest in clay content were most effective in removing protein from suspension.

(5) Decreasing the degree of saturation increased the amount of protein retention as predicted from theoretical considerations.

(6) The soil columns did not retain all the albumin particles. Significant quantities of albumin were always found in the effluent solution. Apparently, aquifers composed predominantly of sands cannot be depended upon to remove all virus particles.

C. Recommendations.

(1) The effect of varying pH should be studied; it seems reasonable to expect the amount of adsorption to be much higher in acid solutions where the albumin is positively charged relative to the soil.

(2) The nature of this retention process should be studied to determine, if possible, the distance over which the adsorptive forces act, the rate of adsorption, the adsorption isotherms, the effect of other chemicals and proteins present in solution and the manner in which proteins other than albumin are retained.

(3) The extent to which experimental results using blood proteins such as albumin can be extrapolated to the case of viruses needs further investigation because the possibility for using these proteins to simulate viruses in full-scale ground water studies is an attractive one. They are not pathogenic and by using short-lived isotopes, field experiments could be made without endangering public health.

(4) Although larger particles such as clays, algae, bacteria and other forms of turbidity may be removed by mechanical sieving or a sedimentation mechanism, the demonstration of an exponential distribution of material with depth does not assure that such is the case, since a similar distribution could be obtained from an adsorptive removal mechanism. Previous results appearing in the literature should be considered with this possibility in mind.

(5) A more sophisticated mathematical analysis is needed which can be applied to recharge of aquifers, etc., and would include the

effects of diffusion, rate of adsorption, hydraulic properties of the soil, the use of a slug of varying thickness and the correct isothermal relationship for the adsorptive mechanism. Unfortunately, much of this information does not appear to be presently available.

(6) Studies should be made using soils containing larger percentages of clay, silt and organic matter than the materials used in this study.

(7) Since virus particles exhibit electrophoretic properties, the possibility of removal by an applied electric field with subsequent precipitation on the electrodes should be investigated. Viruses, if present in processed water, are in very low concentration and such a device would not necessarily be required to remove large quantities.

One way this might be done is by using a specially constructed filter consisting of alternate layers of sand and porous metallic electrodes. Another possibility is that of precipitating clays out on the sand of a filter bed by the method of Rausch and Curry. The clay-coated sand bed could then be used as a final filter to adsorb virus particles. At such time as the clay lost its effectiveness, it might be stripped off by changing the zeta potential and a new layer of clay applied

BIBLIOGRAPHY

Abramson, H. A., 1932, Electrokinetic phenomena. Relationship between electric mobility, charge and titration of proteins. Journal of General Physiology 15: 575 - 603.

Adams, M. H., 1948, Surface inactivation of bacterial viruses and of proteins. Journal of General Physiology 31: 417 - 431.

______, 1959, Bacteriophages. New York: Interscience Publishers, Inc., 592 p.

Adamson, A. W., 1964, Physical chemistry of surfaces. New York: Interscience Publishers, Inc., 629 p.

Alderton, G., W. H. Ward and H. L. Ferold, 1945, Isolation of lysozyme from eggwhite. Journal of Biological Chemistry 157: 43 - 58.

Anderson, T. F., 1950, The destruction of bacterial viruses by osmotic shock, (abstract). Journal of Applied Physics 21: 70.

Bale, W. F. and I. L. Spar, 1954, In vivo localization of rat organ antibodies in ovaries, adrenals and other tissues. Journal of Immunology 73: 125 - 133.

Bodziony, J. and J. Litwiniszyn, 1962, Mathematical approach to phenomenon of colmatage of n-fractional suspension of particles. Academie Polonaise Des Sciences, Bulletin Series Des Sciences Techniques 10 (n. 1): 43 - 49.

Borchardt, J. A. and C. R. O'Melia, 1961, Sand filtration of algal suspensions. American Water Works Association, Journal 53: 1493 - 1502.

Bower, C. A. and J. O. Goertzen, 1959, Surface area of clays and soils by an equilibrium ethylene glycol method. Soil Science 87: 289 - 292.

Brooks, R. H. and A. T. Corey, 1964, Hydraulic properties of porous media. Fort Collins, Colorado: Colorado State University Hydrology Paper No. 3, 27 p.

Burdine, N. T., 1953, Relative permeability calculations from pore-size distribution data. Petroleum Transactions, American Institute of Mining Engineers 198: 71 - 77.

Camp, T. R., 1964, Theory of water filtration. American Society of Civil Engineers, Proceedings 90 (SA4): 1 - 30.

Carlson, H. J., G. M. Ridenour and C. F. McKhann, 1942, Efficacy of standard purification methods in removing poliomyelitis virus from water. American Journal of Public Health 32: 1256 - 1262.

Chang, S. L., R. E. Stevenson, A. R. Bryant, R. L. Woodward and P. W. Kabler, 1958, Removal of coxsackie and bacterial viruses in water by flocculation. American Journal of Public Health 48: 51 - 61.

Corey, A. T., 1954, The inter-relation between gas and oil relative permeabilities. Producer's Monthly 19 (n. 1): 38 - 41.

Curry, R. B. and R. P. Beasley, 1962, The flow of colloidal suspensions through porous media as related to reservoir sealing. American Society of Agricultural Engineers, Transactions 5: 160 - 164.

______, G. L. Barker and Z. Strach, 1964, The interrelationship of physical and chemical properties involved in the flow of colloidal suspensions in porous media. American Society of Agricultural Engineers, Paper No. 64 - 217, 1964 Annual Meeting.

de Boer, J. H., 1953, The dynamical character of adsorption. London: Oxford University Press, 239 p.

Dieterich, B. H., 1951, Behavior of bacterial virus in contact with ordinary and uniform filter sand. Cambridge: Unpublished Master's Thesis, Harvard University, 53 p. (microfilm).

Dummett, A. and P. Bowden, 1933, The influence of the underlying surface on the cataphoretic mobility of adsorbed proteins. Royal Society of London, Proceedings A. 142: 382 - 400.

Francis, G. E., W. Mulligan and A. Wormal, 1951, Labelling of proteins with iodine-131, sulfur-35 and phosphorous-32. Nature: 167: 748 - 751.

Ghosh, G., 1958, Media characteristics in water filtration. American Society of Civil Engineers, Proceedings 84 (SA 1): 1533, 25 p.

Hall, W., 1957, An analysis of sand filtration. American Society of Civil Engineers, Proceedings 83 (SA 3): 1276, 9 p.

Heiple, L. R., 1959, Effectiveness of coarse grained media for filtration. American Water Works Association, Journal 51: 749 - 760.

Ives, K. J., 1961, Filtration using radioactive algae. American Society of Civil Engineers, Proceedings 87 (SA 3): 2820, 15 p.

Jackson, R. D., R. J. Reginato and W. E. Reeves, 1962, A mechanical device for packing soil columns. U. S. Department of Agriculture, A. R. S. 41 - 52, 10 p.

Jorden, R. M., 1963, Electrophoretic studies of filtration. American Water Works Association, Journal 55: 771 - 782.

Kempf, J. E., M. G. Wilson, M. E. Pierce and M. H. Soule, 1942, Effect of aluminum hydroxide, sedimentation, sand filtration and chlorination on the virus of poliomyelitis. American Journal of Public Health 32: 1366 - 1370.

Krone, R. B., G. T. Orlob and C. Hodgkinson, 1958, Movement of coliform bacteria through porous media. Sewage and Industrial Wastes 30 (n. 1): 1 - 13.

Kunitz, M., 1946, Crystalline soybean trypsin inhibitor. Journal of General Physiology 29: 149 - 154.

Litwiniszyn, J., 1963, Colmatage considered as a certain stochastic process. Academie Polonaise Des Sciences, Bulletin Series Des Sciences Techniques 11 (n. 3): 117 - 121.

McKinney, R. E., 1962, Microbiology for sanitary engineers. New York: McGraw-Hill Book Co., Inc., 293 p.

McLean, D. M., 1964, Contamination of water by viruses. American Water Works Association, Journal 56: 585 - 591.

Mosley, J. W., 1959, Water-borne infectious hepatitis. New England Journal of Medicine 261: 703 - 748.

Mysels, K. J., 1959, Introduction to colloid chemistry. New York: Interscience Publishers, 475 p.

Neurath, H. and K. Bailey, 1953, The proteins. New York: Academic Press, Inc., 2 v.

Pollard, E. C., 1953, The physics of viruses. New York: Academic Press, Inc., 230 p.

Rausch, D. L. and R. B. Curry, 1963, The effect of viscosity and zeta potential of bentonite suspensions on flow through porous media. American Society of Agricultural Engineers, Transactions 6: 167 - 169.

Richards, L. A., 1931, Capillary conduction of liquids through porous mediums. Physics 1: 318 - 333.

Robeck, C. G., N. A. Clarke and K. A. Dostal, 1962, Effectiveness of water treatment processes in virus removal. American Water Works Association, Journal 54: 1275 - 1290.

__________, K. A. Dostal and R. L. Woodward, 1964, Studies of modifications in water filtration. American Water Works Association, Journal 56: 198 - 213.

Suter, M., 1956, High rate recharge of ground water by infiltration. American Water Works Association, Journal 48: 355 - 360.

Wyllie, M. R. J. and G. H. F. Gardner, 1958, The generalized Kozeny-Carman equation. World Oil 146: 121, 123 - 126, 210, 214, 216, 219 - 220, 225 - 228.

______, and M. B. Spangler, 1952, Application of electrical resistivity measurements to the problem of fluid flow in porous media. American Association of Petroleum Geologists, Bulletin 36: 359 - 403.

Zvyagintsev, D. G., 1962, Adsorption of microorganisms by soil particles. Soviet Soil Science, February 1962: 140 - 144.

APPENDIX A

A. Experimental Procedure Utilized in this Study for Labeling Bacteriophages.

The procedure used for labeling bacteriophages was that of Bale and Spar (1954). Dialysis was carried out at 4°C against 100 ml. quantities of borate or glycine buffered saline. It was established after exhaustive dialysis that the phage solution inside the cellophane dialyzing sack contained a substantial amount of radioactivity. This was measured and the solution was then spread on agar plates containing E. Coli. After suitable incubation, the plaques were counted and the titer determined.

B. Results.

About 0.4 micrograms of Iodine-131 was incorporated into 1.2 mls. of a T-7 coliphage culture of titer 10^9. This approximate figure is based on the activity of the solution since there was inadequate protein present to permit the normal precipitation procedure. Subsequent tests on the labeled phage revealed that the titer had dropped to 10^3 during the three-day period involved in labeling. To the author's knowledge, this was the first time this type of labeling had been done with bacteriophages. Since the phages could not be concentrated sufficiently to permit their detection through the acrylic permeameters, subsequent studies were made using radio-iodinated human serum albumin.

APPENDIX B

Relative Permeability - Capillary Pressure Data

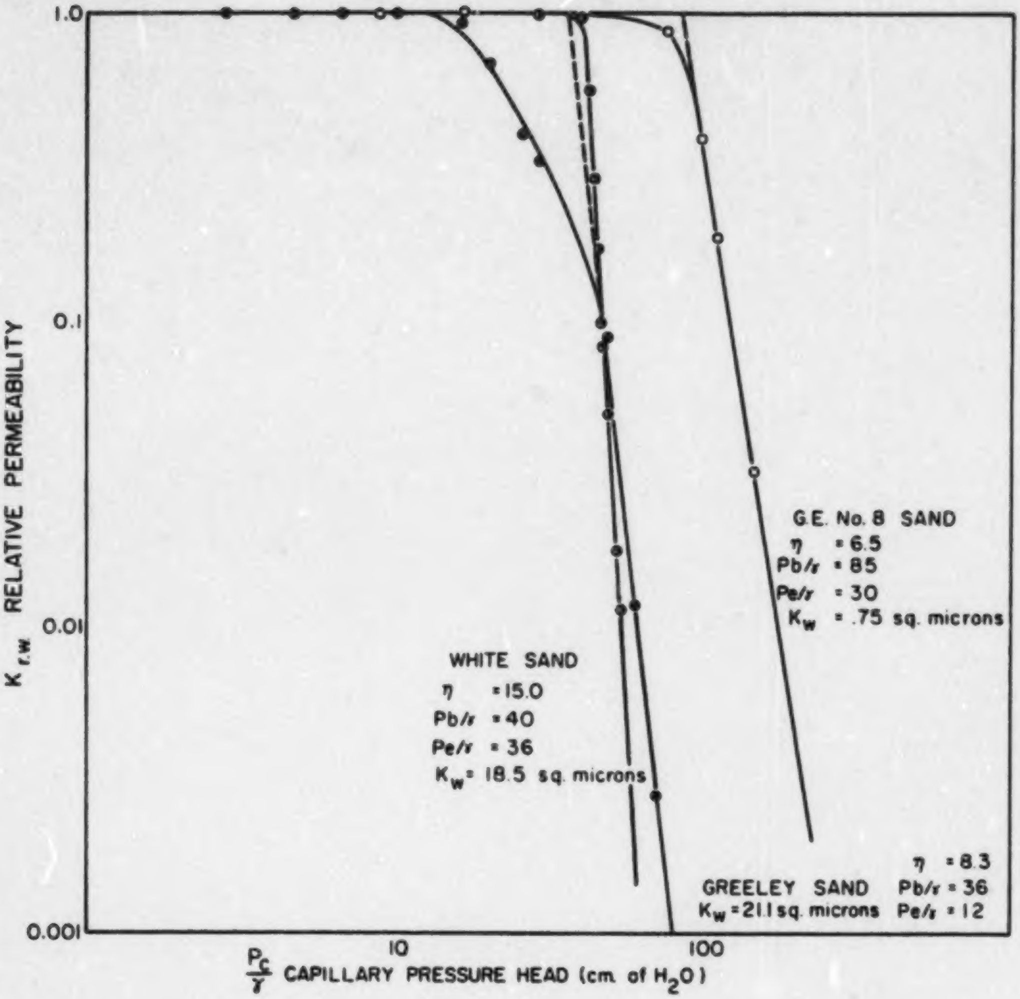


Figure 23. Relative permeability as a function of capillary pressure

APPENDIX C

Capillary Pressure-Saturation Data

This information was supplemental to the main experiment and provided a knowledge of the degree of saturation of the soil for any given capillary pressure at which the permeameter was operating. The data are presented in Figure 14. The apparatus is shown in Figure 25. The sample for this experiment was one of the 4 cm. sections cut from the same permeameter used for the relative permeability determinations. One of the capillary inflow barriers was connected to a horizontal, calibrated tube so that the volume of water drained from the sample could be determined. The barrier was placed in contact with the soil as shown in the sketch and the other end of the calibrated tube was connected to a closed, water-filled reservoir by a piece of flexible plastic tubing. This reservoir was then connected by another plastic tube to a second water-filled reservoir which was open to the atmosphere. This system of reservoirs made it possible to control the capillary pressure in the sample precisely.

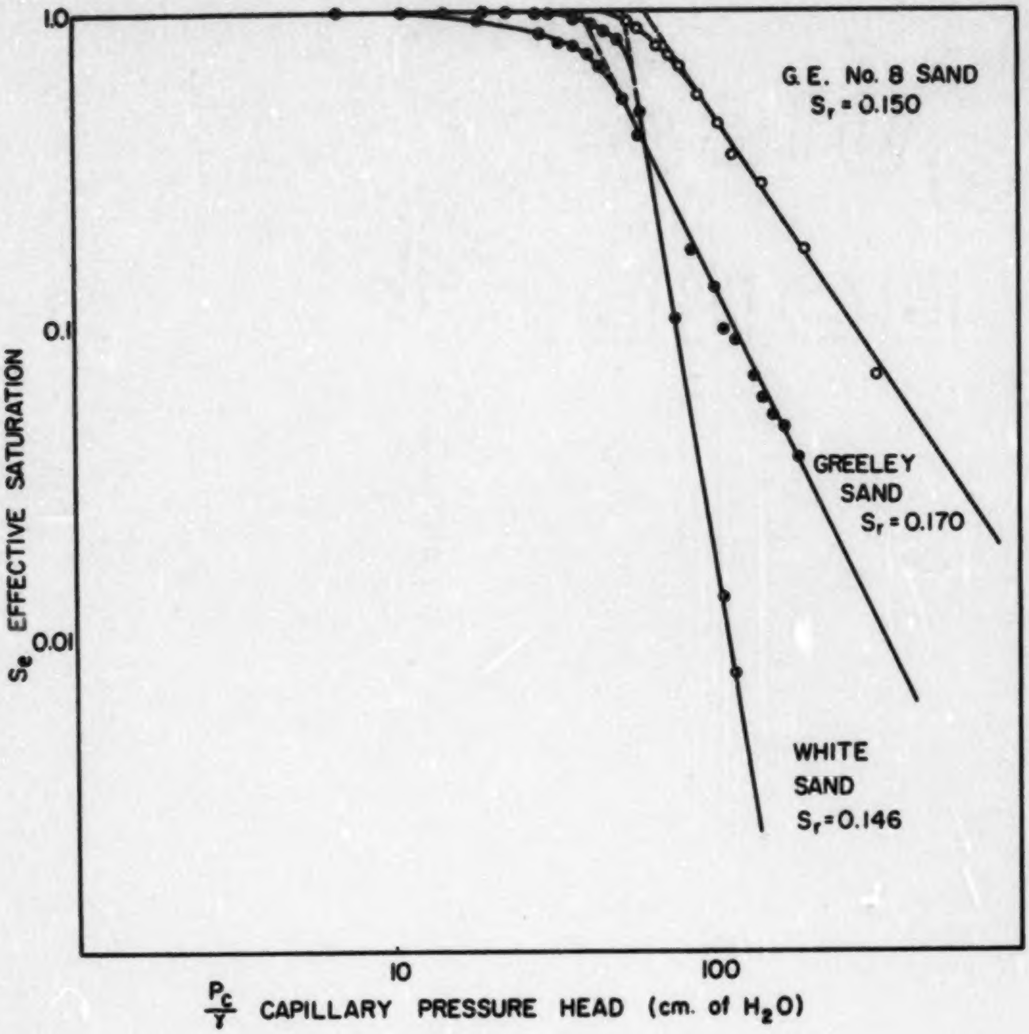


Figure 24. Saturation as a function of capillary pressure

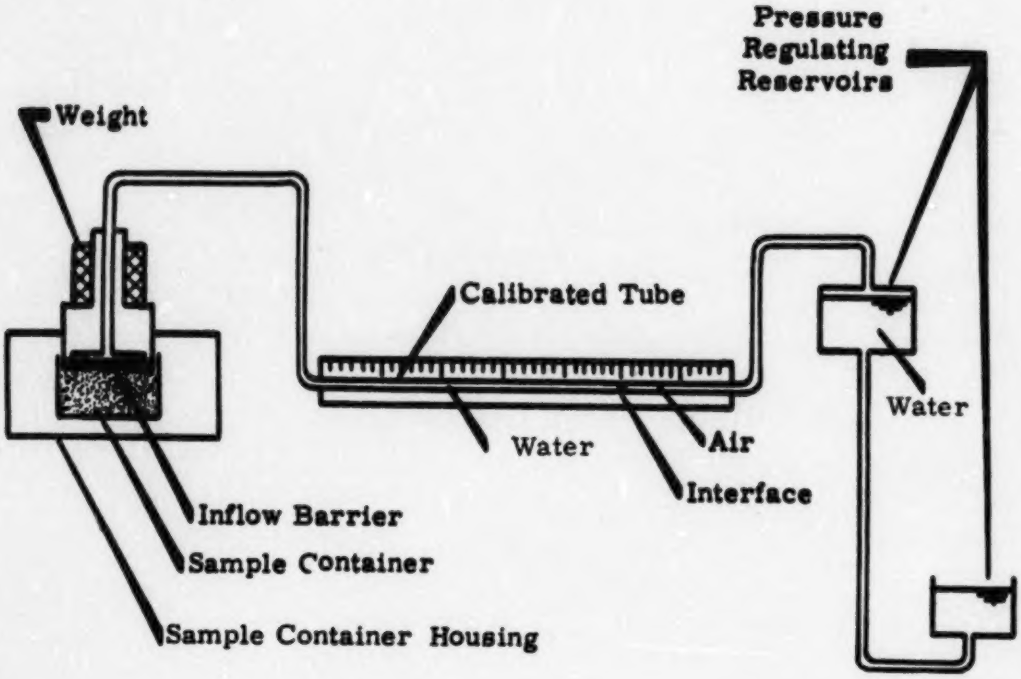

Pressure
Regulating
Reservoirs
Weight
Calibrated Tube
Water
Water
Air
Interface
Inflow Barrier
Sample Container
Sample Container Housing

Figure 25 Apparatus for measuring saturation as a function of capillary pressure

APPENDIX D

Calculation of the Albumin Retained in the Permeameter

Since some of the albumin added was held in the metering device, it was not possible to determine retention by merely sampling the effluent. The amount of material in the permeameter also had to be determined.

Using the data obtained from dissecting the column, a graph was made of activity per sample slice as a function of depth. All measurements were standardized to 6 gm. samples. A smooth curve was drawn through these points and the area under the curve was obtainable graphically. The total activity in the column was then determined by using the following formula:

$$G = \frac{\rho_b A}{6} \int_o^L \theta \, dy$$

where G is the total activity expressed in counts per minute or in milligrams of albumin, ρ_b is the bulk density in gms. per c. c. of soil, A is the cross-sectional area of the column and $\int_o^L \theta \, dy$ represents the area under the activity curve.

APPENDIX E

Run 3 - G. E. No. 8 sand

P_c/γ = 9.0 cm. H_2O

S_e = 1.0

K_w = .75 sq. microns

Iodinated Calf Serum
(quantity not known)

y (cm.)	Mean One Minute count x 10^{-3}
1.0	3.17
5.5	3.52
7.5	1.43
11.5	1.18
15.5	0.51
17.5	0.18
21.5	0.06
25.5	0.08
27.5	0.07
31.5	0.06
35.5	0.10
37.5	0.03
41.5	0.04

Run 4 - G. E. No. 8 sand

P_c/γ = 18.0 cm. of H_2O

K_w = .97 sq. microns

S_e = 1.0

1.38 mg. of I. H. S. A.

92% retention

y (cm.)	Mean 30-Second count x 10^{-3}
0	11.9
1	338.0
2	170.5
3	142.2
4	138.4
5	117.0
6	93.5
7	85.3
8	59.0
9	42.8
10	54.7
11	26.2
12	13.3
13	4.8
14	0.49
15	0.35
16	0.27
17	0.29
18	0.24
19	0.23
20	0.36
21	0.37
22.5	0.40
23.5	0.41
25.5	0.90
26.5	0.75
27.5	0.21

Run 5 - G. E. No. 8 sand

P_c/γ = 20 cm. H_2O

K_w = .75 sq. microns

S_e = 1.0

5.85 mg. I. H. S. A.

96% retention

y (cm.)	Mean 30-Second count x 10^{-3}
0.6	102.7
2.25	69.6
2.75	57.9
3.25	42.6
4.75	35.3
5.25	29.4
5.75	30.9
6.25	27.3
6.75	27.4
7.25	21.0
7.75	13.7
9.25	9.3
9.75	9.7
10.25	7.2
10.75	4.9
13.25	2.3
14.75	1.7
15.25	0.8
18.75	0.2
22.75	0.05
25.25	0.04
28.75	0.04

Run 6 - Greeley sand

P_c/γ = 11.5 cm. H_2O

K_w = 10.7 sq. microns

S_e = 1.0

5.5 mg. of I. H. S. A.

87% retention

y (cm.)	Mean 30-Second count x 10^{-3}
0.00	116.6
1.25	76.7
2.25	64.6
3.25	41.3
5.25	20.0
6.25	14.4
6.75	11.7
8.00	6.7
9.25	6.8
10.25	5.4
12.75	4.2
14.75	4.3
19.25	3.0
22.75	2.3
24.75	4.2
25.25	5.0
25.75	3.8
28.75	0.17

Run 7 - Greeley sand

P_c/γ = 4.0 cm. H_2O

K_w = 12.7 sq. microns

S_e = 1.0

Pre-treated with .05% Calf Serum

5.5 mg. I. H. S. A.

51% retention

y (cm.)	Mean 30-Second count x 10^{-3}
1.25	12.3
2.25	19.1
2.75	19.5
4.75	8.8
7.00	6.1
9.25	1.4
9.75	1.6
10.25	1.8
10.75	1.8
13.25	0.4
15.25	0.4
19.25	0.4
19.75	0.5
23.25	0.3
25.25	0.1

Run 8 - Greeley sand

P_c/γ = 13.5 cm. H_2O

K_w = 11.7 sq. microns

S_e = 1.0

Pre-treated with Human Serum Albumin
5.5 mgs. I. H. S. A.

46% retention

y (cm.)	Mean 30-Second count x 10^{-3}
.25	83.0
1.25	135.0
1.75	124.0
2.45	110.0
3.95	63.9
5.75	24.0
6.45	21.4
7.15	17.6
7,85	17.4
9.05	9.9
9.75	8.2
10.45	8.6
11.15	10.3
11.85	6.8
13.95	6.8
15.85	6.6
16.55	5.5
17.25	4.8
17.95	4.7
19.15	4.5
20.55	3.9
21.95	5.7
23.95	6.2
26.35	1.1
27.85	1.0

Run 9 - White sand

P_c/γ = 7.0

K_w = 18.1 sq. microns

S_e = 1.0

Pre-treated with Human Serum Albumin
2.4 mgs. of I.H.S.A.

18% retention

y (cm.)	Mean 30-Second count x 10^{-3}
.25	8.6
.90	5.6
1.80	8.2
2.55	9.6
4.05	8.8
4.55	9.3
5.55	8.5
6.55	7.1
7.55	7.4
8.15	6.4
8.65	6.7
9.15	6.8
12.15	7.9
14.15	11.2
14.65	9.9
15.15	10.7
15.65	11.1
16.15	11.2
18.20	11.8
18.75	13.3
19.25	14.8
21.75	16.4
22.25	13.7
22.75	13.7
24.25	14.4
24.75	15.2
25.25	14.4
27.25	16.6
28.25	12.7
28.75	15.1
29.75	15.3
30.75	14.6
32.25	10.4
32.75	10.5

Run 10 - White sand

P_c/γ = 46 cm. H_2O

K_w = 14.7

K_{rw} = .083

Pre-treated with Calf Serum
and Human Serum Albumin
2.4 mgs. I. H. S. A.

75.5% retention

y (cm.)	Mean 30-Second count x 10^{-3}	y (cm.)	Mean 30-Second count x 10^{-3}
		cont.	
.25	6.3	40.55	6.0
.75	11.0	42.55	7.7
1.85	18.8	44.55	5.9
2.35	23.0	46.55	6.0
2.85	26.6	48.55	4.3
4.45	24.8	49.55	5.4
4.90	25.0	54.55	4.8
5.85	31.6	56.55	4.0
7.95	37.0	60.55	2.5
8.45	32.8	64.55	1.5
9.45	35.2	68.55	1.4
10.45	34.5	72.55	0.9
11.45	34.4	80.55	0.2
12.95	28.6		
14.45	24.1		
16.45	23.9		
17.45	22.3		
18.45	22.4		
20.55	23.1		
21.55	20.9		
22.55	17.4		
24.55	12.4		
27.55	13.9		
28.55	10.7		
31.55	12.4		
32.55	12.3		
34.55	12.1		
36.55	10.3		
38.55	9.8		

Run 11 - White sand

P_c/γ = 10.0 cm. H_2O

K_w = 15 sq. microns

S_e = 1.0

Pre-treated with Calf Serum and Human Serum Albumin
2.4 mgs. of I. H. S. A.

35% retention

y (cm.)	Mean 30-Second count x 10^{-3}
0.4	8.9
1.05	19.2
1.55	27.7
2.05	36.3
2.55	31.3
3.05	29.9
4.55	23.6
5.05	18.9
6.05	14.3
7.05	10.7
8.05	7.2
9.05	4.4
10.05	5.1
11.05	6.0
13.05	4.7
15.05	5.0
16.05	6.2
17.05	5.7
19.05	3.6
20.05	6.4
21.05	6.4
22.05	5.3
23.05	4.2
24.05	4.6
25.05	3.7
26.05	6.3
27.05	8.6
28.05	8.9
29.05	7.2
30.05	6.8
31.05	7.8
32.05	6.1

Run 12 - White sand

P_c/γ = 12.0 cm. H_2O	2.4 mgs. of I. H S. A.
K_w = 18.5 sq. microns	58% retention
S_e = 1.0	

y (cm.)	Mean 30-Second count x 10^{-3}
.25	11.2
.75	18.9
1.25	32.8
1.75	48.7
4.20	20.7
5.45	13.7
6.45	12.1
7.45	10.2
8.45	11.5
9.45	7.9
10.45	5.4
11.45	6.4
12.45	5.7
13.45	3.8
14.45	5.4
15.45	4.8
16.45	4.2
17.45	4.2
18.45	4.2
19.45	4.7
20.70	4.1
21.45	3.6
23.45	4.3
25.45	3.0
27.45	3.8
28.75	2.8
30.75	3.2
32.25	3.9

Run 13 - White sand

P_c/γ = 53.0 cm. H_2O

K_w = 11.9 sq. microns

K_{rw} = .01

Pre-treated with Calif Serum and Human Serum Albumin
2.4 mg. of I. H. S. A.

93% retention

y (cm.)	Mean 30-Second count x 10^{-3}
.25	4.6
.75	6.7
1.25	12.4
1.75	21.5
2.25	30.0
2.75	29.0
3.85	22.2
4.85	13.7
5.85	8.8
6.85	7.4
7.85	6.2
8.85	4.6
9.85	4.0
10.85	3.4
11.85	2.6
13.75	1.1
16.00	0.47
17.00	0.42
18.00	0.35
19.00	0.34
20.00	0.33
21.00	0.23
22.00	0.28
23.00	0.23
25.25	0.17
27.25	0.10
29.25	0.09
31.25	0.06
33.25	0.08
33.75	0.07
35.75	0.05
37.75	0.06
39.75	0.04
41.75	0.03
43.75	0.02
47.25	0.05

Run 14 - White sand

P_c/γ = 42 cm. H_2O

K_w = 17.7 sq. microns

K_{rw} = .035

See Chapter IV for Description of Procedure

y (cm.)	Mean 30-Second count x 10^{-3}
.25	26.0
.75	17.0
1.25	15.0
1.75	12.0
2.75	9.9
4.75	8.4
6.75	7.7
8.75	8.0
10.75	6.8
12.75	6.2
14.75	5.7
16.75	5.4
18.75	5.2
20.75	5.3
22.75	5.3
24.75	5.2
26.75	5.4
28.75	5.6
30.75	5.7
32.75	5.0
34.75	5.2
36.75	4.9
38.75	4.7
40.75	4.6
42.75	5.2
44.75	4.3

Run 15 - Greeley sand

P_c/γ = 4.0 cm. H_2O 700 mg. I. H. S. A.

K_w = 22.0 sq. microns 28% retention

S_e = 1.0

y (cm.)	Mean 30-Second count x 10^{-3}
.25	91.5
.75	81.4
1.25	75.0
1.75	72.5
2.25	69.0
3.25	67.5
4.25	70.6
5.75	69.0
7.25	70.5
8.25	71.0
9.25	72.4
10.25	68.6
11.25	68.5
12.25	65.5
13.25	61.0
14.25	64.6
15.25	62.4
16.25	62.7
17.25	61.1
18.25	62.0
19.25	54.2
20.25	54.0
21.25	50.7
22.25	48.8
23.25	49.7
24.25	52.4
26.25	51.8
27.25	49.6